INVESTIGATING RARE AND ENDEMIC POLLUTION-SENSITIVE SUBTERRANEAN FAUNA OF VULNERABLE HABITATS IN THE NCR

FINAL REPORT

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EXECUTIVE SUMMARY

Two kinds of shallow groundwater habitat are known to occur in the lower Potomac drainage. Hyporheic zones are water filled, interstitial spaces in alluvial deposits along stream channels. Hypotelminorheic habitats, commonly called seeps, are shallow aquifers perched above a clay layer and characterized by a small drainage. Previous studies have shown that seeps in the lower Potomac drainage are biologically diverse. Currently, seven species of stygobionts (aquatic, subterranean species) are known from seeps near Washington D.C. These include 5 species of amphipod in the genus *Stygobromus,* the isopod, *Caecidotea kenki,* and the snail, *Fontigens bottimeri.*

In this study, 72 seeps and 16 hyporheic sites were identified from six national parks (George Washington Memorial Parkway, Manassas National Battlefield Park, National Capital Parks – East, Prince William Forest Park, Wolf Trap National Park for the Performing Arts, and Chesapeake and Ohio Canal National Historical Park) and two Fairfax Co. VA parks (Riverbend Park and Scott's Run Nature Preserve). In addition, data on 53 previously sampled seeps were also used for analysis.

Stygobionts were collected from 62 seeps (49.2%), and *Stygobromus* was collected from 49 seeps (38.9%). Stygobionts were not found at hyporheic sites. Physicochemical parameters were measured for hyporheic sites and seeps. Seeps had significantly lower mean pH ($p < 0.005$) and higher dissolved oxygen ($p = 0.01$) than hyporheic sites. Significant physicochemical differences between seeps with and without stygobionts were not found. Seeps with *Stygobromus* species had a lower mean pH than seeps without *Stygobromus* (uncorrected $p = 0.01$). Discriminant equations were derived to predict the presence of stygobionts and *Stygobromus* using physicochemical data but had relatively poor predictive power.

Significant seeps were identified based on the presence of multiple species of stygobionts, the presence of rare species, or large populations of stygobionts. Impacted or potentially impacted seeps were also identified along with major threats. The most commonly identified threats to seeps were soil compaction and contamination from pesticides, herbicides, or road runoff.

INTRODUCTION

97% of all unfrozen freshwater is subsurface water (Gibert, Danielopol, and Stanford, 1994). Numerous groundwater habitats have been identified, each with distinct physical and chemical characteristics and unique and specialized fauna. The species that are limited to groundwater habitats are called stygobionts, and they have unique morphological and physiological adaptations including eye loss, pigment loss, and decreased metabolic rate. In addition to these “regressive” characteristics, stygobionts have evolved a variety of extra-optic sensory structures and attenuated appendages to effectively move, find food, and avoid predators in an aphotic environment. For example, elongated legs and antennae are common features of both aquatic and terrestrial subterranean species. Abundant, elongated setae are common in stygobiontic arthropods, and stygobiontic fish have extra-sensitive lateral lines and olfactory receptors.

Most stygobionts are limited to a specific groundwater environment and are relatively sensitive to environmental variation (i.e. they are stenophiles). For example, deep aquifers such as the Edwards Aquifer in Texas (deep groundwater), and underground streams in caves have very different biotic assemblages. Groundwater habitats do not only occur at great depths. Groundwater habitats very near the surface can still harbor a rich fauna with morphological adaptations to a subterranean lifestyle. For example, epikarst is the transitional zone between soil and caves which is only partially saturated with water and often rivals other cave environments in terms of biodiversity (Pipan, 2005). The habitats discussed so far all occur in karst – areas underlain by soluble rock such as limestone or gypsum. However, groundwater habitats also occur in non-karstic areas and harbor an equally rich fauna. For example, the water-filled spaces between unconsolidated sediments, soil, leaf-litter, and rocks provide a complex habitat for a variety of subterranean species. These habitats are called interstitial habitats and are some of the most superficial of groundwater habitats. Because of their superficial nature, these habitats have higher nutrient inputs and greater physicochemical variability than deeper groundwater habitats. They are also potentially more susceptible to anthropogenic disturbances.

This study surveys the fauna and chemical parameters of two of these superficial groundwater habitats: the hypotelminorheic and hyporheic zones. Hypotelminorheic habitats, or seeps, are shallow aquifers in soil perched above a layer of clay whereas hyporheic zones are the interstitial spaces in alluvial deposits along stream and river channels.

The interstitial groundwater fauna of seeps and hyporheic zones of the lower Potomac drainage is unique, exceptionally diverse, and vulnerable to a variety of anthropogenic impacts. Hyporheic zones have been better studied than seeps, but previous work suggests that in the lower Potomac drainage, seeps contain the greatest diversity of subterranean species.

The purpose of this study is to identify and sample previously sampled and unsampled seeps and hyporheic habitats in six national parks of the Capital Region. These parks include the George Washington Memorial Parkway (GWMP), Manassas National Battlefield Park (MANA), National Capital Parks – East (NACE), Prince William Forest Park (PRWI), Wolf Trap National Park for the Performing Arts (WOTR), and Chesapeake & Ohio Canal National Historical Park (CHOH) between Washington DC and Great Falls. In addition to these parks, seeps in two additional Fairfax County parks, Riverbend and Scott's Run, were also sampled. New localities for species were identified in order to better understand the distribution of species as well as the biodiversity of superficial groundwater habitats in the lower Potomac drainage. Furthermore, chemical characteristics were recorded for each sampling location. These data are used to better characterize hypotelminorheic and hyporheic sites and distinguish seeps and hyporheic sites yielding stygobionts from sites without stygobionts. This information compliments previous research that has been conducted in the lower Potomac drainage. This report also identifies particularly rich sites in terms of number of species, sites containing rare species, and sites with high population densities for one or more species of stygobiont. Finally, at risk seeps are identified, and management recommendations are given for these sites.

1.1 SEEPS AND HYPORHEIC ZONES

The Croatian biologist Milan Meštrov (1962) was the first to describe seeps, which he referred to as the "hypotelminorheic." In appearance, seeps may look similar to vernal pools, puddles of rainwater, or small springs (Fig. 1). However, Culver *et al.* (2006) list several physical characteristics that distinguish seeps from other, superficially similar habitats.

1. A persistent wet spot, a kind of perched aquifer.
2. Fed by subsurface water in a slight depression in an area of low to moderate slope.
3. Rich in organic matter
4. Underlain by a clay layer typically 5 to 50 cm beneath the surface.
5. With a drainage area typically of less than 10,000 m^2
6. With a characteristic dark color derived from decaying leaves which are usually not skeletonized.

In addition to these characteristics, seeps may also have higher dissolved oxygen content than many other small water bodies. These characteristics may not hold in all instances (for example some seeps may go dry on the surface, and seeps that are not in wooded areas may not have a dark color), but they do serve as useful guidelines for identifying potential seeps. Several of these characteristics make seeps especially unique and interesting. Because of their small drainage area, seeps are isolated from the water table and from one another, possibly affording opportunities for speciation. Furthermore, the clay layer on which these aquifers are perched may also retain water during periods of drought thus serving as a refuge to groundwater invertebrates. Finally, relative to other subterranean groundwater habitats, seeps are relatively rich in organic matter.

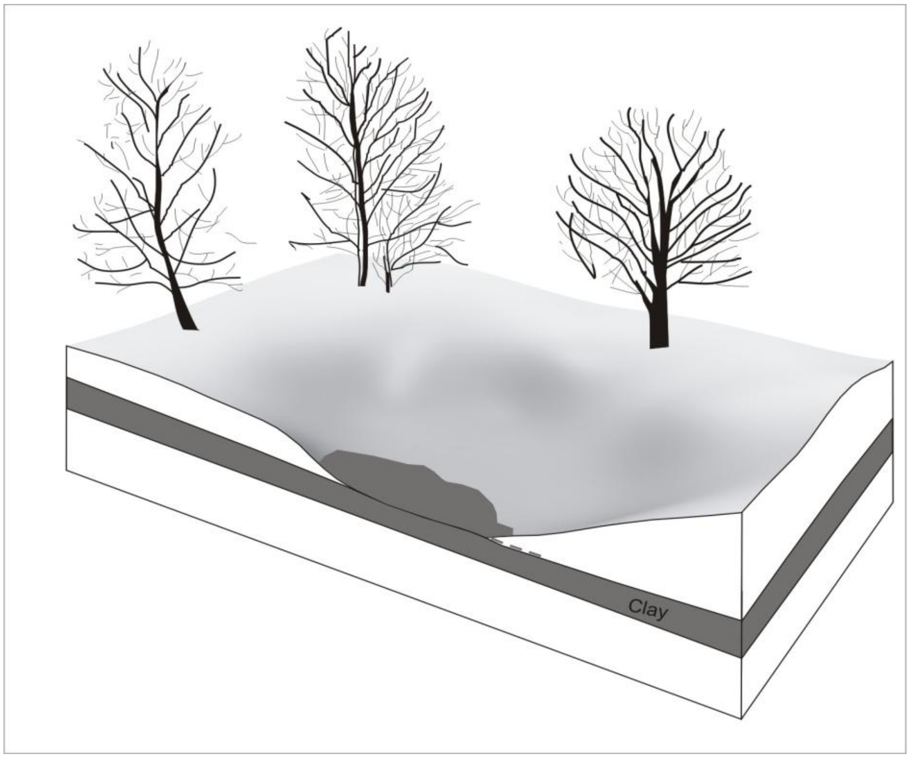

Figure 1: Cross sectional illustration of a seep. Note clay layer which retards downward movement of water. Sketch by Tanja Pipan.

White (1993) defined the hyporheic zone as “the saturated interstitial areas beneath the stream bed and into the stream banks that contain some proportion of channel water, or that have been altered by channel water infiltration.” Malard (2003) also notes that the hyporheic zone may serve as a point of interaction between surface and ground water via upwelling and downwelling. The author defines three types of hyporheic zones differing from one another by the presence or absence of groundwater, connectivity between the hyporheic zone and the groundwater zone, and the lateral extent of the hyporheic zone away from the stream channel (Fig. 2).

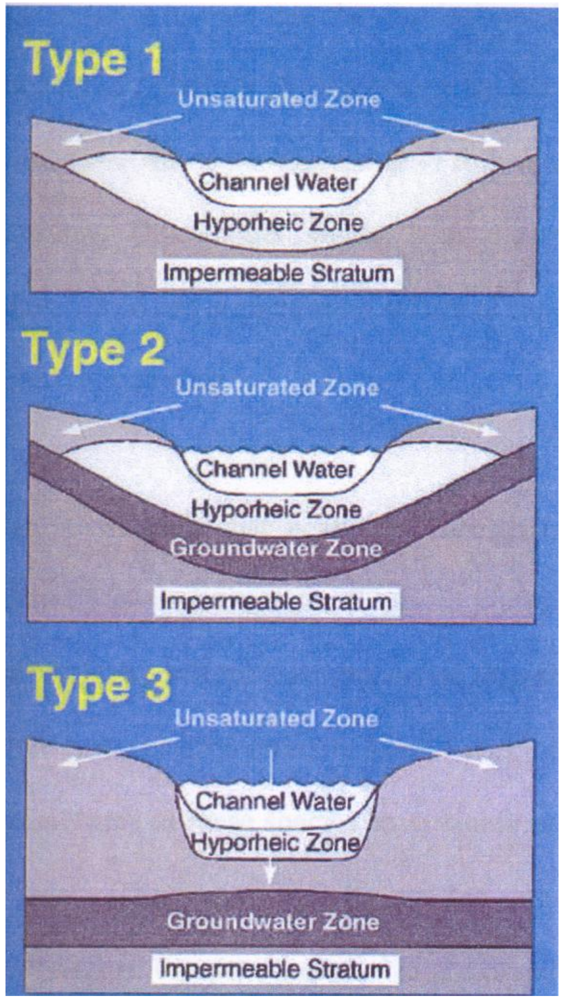

Figure 2: Three types of hyporheic zones (Malard, 2003).

1.2 THREATS

The small size and superficial nature of interstitial habitats puts these ecosystems at risk from a variety of anthropogenic impacts. Furthermore, thin exoskeletons, small population sizes, poor dispersal ability, and slow reproductive potential (common characteristics of many stygobionts) may make stygobiontic interstitial species slow to recover from disturbances. Five major threats to seeps and hyporheic habitats are identified (Culver and Šereg, 2004).

1. Point-source inputs of aquifer contamination from spills (e.g., oil, gas, antifreeze, etc)

2. Non point-source inputs of aquifer contamination from impervious urban surfaces and poor storm water management (e.g., heavy-metals, salt) or other land use (e.g., fertilizers, herbicides, insecticides, sewer leaks, etc)

3. Reduction in recharge of aquifer due to increased impervious urban surfaces or redirection of rainwater.

4. Isolation of separate seeps by impervious urban surfaces or redirection of rainwater.

5. Soil compaction from farming equipment, mowing equipment, or foot traffic.

1.3 THE FAUNA OF LOWER POTOMAC HYPOTELMINORHEIC AND HYPORHEIC HABITATS

The superficial nature of seeps allows for the presence of both stygobionts and species commonly found in surface water habitats. In this study, epigean species are referred to as accidentals. Accidentals are surface species that are sometimes found in subterranean habitats but do not exhibit adaptations to subterranean habitats. Subsequently, populations of accidentals typically cannot persist in subterranean habitats. However, because of the superficial nature of seeps, accidentals are relatively common compared with other subterranean habitats. Furthermore, because seeps share many characteristics with other aquatic surface habitats, accidentals may be fairly successful in seeps. In addition to stygobionts and accidentals, some of the animals inhabiting seeps can be referred to as stygophiles. Stygophiles are not restricted to subterranean habitats, but viable populations of stygophiles can persist in subterranean habitats. Furthermore, these species may show some morphological adaptations intermediate between surface and subsurface species. All of the stygobionts or seep specialists identified in this study belong to three taxa: amphipods, isopods, and gastropods. The species identified in this study, belonging to these three taxa, are listed in table one along with their status as stygobionts, stygophiles, or accidentals. Table one also identifies those species that are seep specialists.

1.3.1 Amphipods

Amphipods dominate the interstitial fauna of the lower Potomac drainage, and three genera (*Stygobromus*, *Crangonyx*, and *Gammarus*) commonly occur in seeps. Of these genera, only *Stygobromu*s is represented by stygobiontic species. Subsequently, *Stygobromus* is one of the focal genera of this study.

1.3.1.1 *Stygobromus*

The genus *Stygobromus* is a large one, being comprised of over 100 described species across much of North America as well as a single species from Siberia and a single species which occurs near the west bank of the Caspian sea (Holsinger, 1987) .

All of the known species of *Stygobromus* are stygobionts, and are known from caves, epikarst, wells, springs, hyporheic zones, and seeps. Like many obligate subterranean species, *Stygobromus* are eyeless, without pigment, and typically have attenuated appendages (Fig. 3). Furthermore, *Stygobromus* species have lower metabolic rates than there surface relatives (Culver and Poulson, 1971). Currently, six species of *Stygobromus* are known from the lower Potomac drainage. All of these species are stygobionts, and three of them are seep specialists. One species, *Stygobromus phreaticus* is not known to inhabit hyporheic or hypotelminorheic habitats. Three species, *Stygobromus hayi* (Hubricht and Mackin), *S. kenki* Holsinger, and *S. phreaticus* Holsinger were not collected from any locations sampled during this study.

Figure 3: *Stygobromus tenuis potomacus.* Photo by William K. Jones.

***Stygobromus tenuis potomacus* (Holsinger)** is the most widespread stygobiont in the lower Potomac drainage. Males reach 16.5 mm in length and females reach 9.5 mm. The range of this subspecies extends from south-central Pennsylvania south to Richmond, Virginia (Holsinger, 1978). The closely related *Stygobromus tenuis tenuis* ranges from central Connecticut and southeastern New York southwest to the Piedmont of eastern Maryland (Holsinger, 1978). *S. tenuis potomacus* is an inhabitant of shallow groundwater and is primarily found in seeps (Holsinger, 1978). The species is also known to occur in springs and shallow wells.

Stygobromus pizzinii **(Shoemaker)** is rarer than *S. tenuis potomacus* and is more patchily distributed. It is the largest species of *Stygobromus* that occurs in the lower Potomac drainage. Males reach 18.7 mm and females reach 15.7 mm. The range of this species extends from southeastern Pennsylvania southwest to Fairfax County, Virginia (Holsinger, 1978). *S. pizzinii* inhabits a variety of groundwater habitats including seeps, springs, wells, and caves (Holsinger, 1978).

Stygobromus hayi **(Hubricht and Mackin)** is a seep specialist and one of only three federally endangered species known from the District of Columbia (the other two being the panther and the bald eagle). Males of this species reach 9.75 mm in length and females reach 10.0mm. *Stygobromus hayi* is known from its' type locality in the National Zoological Park in Washington DC and six seeps and small springs in Rock Creek Park Washington DC. *S. hayi* is known only from seeps associated with Precambrian rocks of the Piedmont (Holsinger, 1967).

Stygobromus kenki **Holsinger** is another seep specialist that has been found in even fewer sites than *S. hayi*. Males reach 3.7 mm in length and females reach 5.5 mm in length. *S. kenki* is known from only four sites in Rock Creek Park, Washington DC. *S. kenki* was previously reported from a 12 m deep well in Fairfax County, Virginia on Edsall Rd which is now destroyed. The specimen collected from this site was immature and not definitively identified (Culver and Šereg, 2004; Holsinger, 1978). Rock Creek Park sites are seeps and small, seep-like springs. Almost nothing is known about the biology of this species.

Stygobromus sextarius **Holsinger, in prep**, previously *Stygobromus sp. 15*, is also a seep specialist previously known from eight seeps in the National Zoological Park, Washington, DC, C & O Canal National Historic Park, Maryland, Turkey Run Park in Fairfax, Va, the George Washington Memorial Parkway, VA, and in Rock Creek Park, Washington, D.C. (Culver and Šereg, 2004).

***Stygobromus phreaticus* Holsinger** is not found in hyporheic and hypotelminorheic habitats, but inhabits deeper groundwater in the lower Potomac drainage. Males reach 6.8 mm in length and females reach 7.0 mm in length. *S. phreaticus* is known from two wells in Vienna and Alexandria, Virginia (Holsinger, 1978).

1.3.1.2 *Crangonyx*

Although no stygobiontic *Crangonyx* species occur in interstitial habitats of the lower Potomac Drainage, several species can be found in seeps, sometimes occurring with *Stygobromus* species. Two of these species, *Crangonyx floridanus* Bousfield and *C. serratus* (Embody) were not collected from any locations sampled during this study.

***Crangonyx shoemakeri* (Hubricht and Mackin)** is by far, the most common *Crangonyx* species found in seeps in the lower Potomac drainage. Males of this eyed, pigmented species reach 9.5 mm in length and females reach 13.5 mm. This species ranges from south-central Maryland south to south-central Virginia in the Blue Ridge, Piedmont, and Coastal Plain (Zhang and Holsinger, 2003). *C. shoemakeri* inhabits seeps, springs, bogs, ponds, small streams, and temporary pools (Zhang and Holsinger, 2003).

***Crangonyx stagnicolous* Zhang and Holsinger** is a recently described species**.** Males reach 5.2 mm in length and females reach 9.0 mm in length (Zhang and Holsinger, 2003). This species is found in the coastal plain of southern Maryland, and the western edge of the coastal plain in Fairfax Co. VA (Zhang and Holsinger, 2003). *C. stagnicolous* is primarily found in swamps, ponds, and small pools, but has also been collected from seeps (Zhang and Holsinger, 2003).

***Crangonyx palustris* Zhang and Holsinger** is another recently described species. Males reach 5.5 mm and females reach 9.0 mm (Zhang and Holsinger, 2003). This species is found primarily in the coastal plain between west-central New Jersey and the extreme north-eastern corner of North Carolina. A few specimens have also been collected above the fall line immediately south west of Washington, D.C. (Zhang and

Holsinger, 2003). *C. palustris* is found in seeps, swamps, springs, ponds, pools, streams, ditches (Zhang and Holsinger, 2003).

***Crangonyx floridanus* Bousfield** is the most widely distributed species of *Crangonyx* that may be found in seeps of the lower Potomac drainage. Males of this species reach 8.0 mm in length and females reach 12.0 mm (Zhang and Holsinger, 2003). This species occurs across much of the eastern and east-central United States from Massachusetts to southern Florida and west to central Kansas (Zhang and Holsinger, 2003). *C. floridanus* inhabits swamps, ponds, streams, and cave pools as well as springs and seeps in the lower Potomac drainage (Zhang and Holsinger, 2003).

***Crangonyx serratus* (Embody).** Males of this species reach 11.0 mm and females reach 16.0 mm. This species is found almost entirely in the coastal plain between Washington, DC and north-central Florida (Zhang and Holsinger, 2003). *C. serratus* inhabits seeps, springs, ditches, ponds, and small streams (Zhang and Holsinger, 2003).

1.3.1.3 *Gammarus*

Like *Crangonyx*, no stygobiontic species of the genus *Gammarus* are known to exist in interstitial habitats of the lower Potomac drainage, but two epigean species can be found in seeps.

***Gammarus fasciatus* Say** is a wide ranging, habitat generalist. Males reach 14.0 mm in length and females reach 12.0 mm in length (Holsinger, 1972). This species ranges from the great lakes, across much of New York and south along the coastal plain to South Carolina. *G. fasciatus* can be found in lakes, rivers, streams, and occasionally, springs and seeps (Holsinger, 1972).

***Gammarus minus* Say** is primarily found in karst landscapes, but can also be found in some of the lower Potomac drainage. Males of this species reach 14.0 mm in length and females reach 12.0 mm in length (Holsinger, 1972). This species ranges across much of the Appalachian Mountains, Interior Low Plateau, and the Ozarks (Holsinger, 1972). *G.*

minus is commonly found in springs and spring runs, but can also be found in caves and rarely, seeps.

1.3.2 Isopods

Isopods are another major component of the specialized seep fauna in the lower Potomac drainage and are represented by a single genus: *Caecidotea* (Fig. 4).

1.3.2.1 *Caecidotea*

A single species of *Caecidotea, C. kenki* (Bowman) is a stygobiont and seep specialist. A few others species may occasionally occur in seeps. *Caecidotea* is a large genus in need of taxonomic revision. Some currently recognized species may by conspecific while other currently recognized species may actually be comprised of multiple species. Furthermore, numerous species await description. Consequently, identification of individuals of this species is tentative. Identifications for this study are based on the key given by Williams (1972).

Figure 4: *Caecidotea kenki.* Photo by William K. Jones.

***Caecidotea kenki* (Bowman)** is the only isopod known from the lower Potomac drainage which is considered to be a stygobiont. Individuals of this species reach a length of 10mm. This species is known from southern Pennsylvania, Maryland, Northern Virginia, and Washington DC in the coastal plain and eastern Piedmont (Williams, 1972). *C. kenki* inhabits small springs, small spring-fed creeks, and seeps (Williams, 1972), all of which are superficial groundwater habitats.

***Caecidotea nodulus* (Williams)** is similar in appearance and size (around 10mm) to *C. kenki*. The major distinguishing characteristic is the male 2^{nd} pleopod. The species is known from Maryland, Washington DC, and north-eastern Virginia (Williams, 1972). This species inhabits swamps, ditches, streams, springs (Williams, 1972), and seeps and has small but distinct eyes (Williams, 1970).

1.3.3 Gastropods

A single species of stygobiontic snail in the genus *Fontigens* is known to inhabit seeps of the lower Potomac drainage (Fig. 5).

1.3.3.1 *Fontigens*

Fontigens is a small group of snails distributed across the Appalachian Mountains, Ozark Plateau, and Central Lowlands (Hershler *et al.* 1990). *Fontigens* species inhabit a variety of aquatic habitats including cave streams, springs, spring-fed streams and small lakes (Hershler *et al.* 1990).

***Fontigens bottimeri* (Walker)** is a small species reaching no more than 3mm in height. This species always exhibits some degree of pigment loss like many stygobiontic species. The species range extends from central Maryland south through Washington, D.C. to Frederick County, Virginia (Hershler *et. al.* 1990). The species is known from caves, springs (Hershler *et. al.* 1990), and seeps.

Figure 5: Fontigens bottimeri. Photo by William K. Jones.

Table 1: Species inhabiting superficial groundwater habitats identified in this study.

Genus	Species	Styogiont, Stygophile, or Accidental	Seep Specialist
Amphipods			
Stygobromus	*tenuis potomacus*	stygobiont	yes
	pizzinii	stygobiont	no
	hayi	stygobiont	yes
	kenki	stygobiont	yes
	sextarius	stygobiont	yes
	phreaticus	stygobiont	no
Crangonyx	*shoemakeri*	stygophile	no
	stagnicolus	accidental	no
	palustris	accidental	no
	floridanus	stygophile	no
	serratus	accidental	no
Gammarus	*fasciatus*	accidental	no
	minus	stygophile	no
Isopods			
Caecidotea	*kenki*	stygobiont	yes
	nodulus	stygophile	no
Gastropods			
Fontigens	*bottimeri*	stygobiont	yes

METHODS AND MATERIALS

2.1 STUDY AREA

Hypotelminorheic and hyporheic sites were sampled in six national parks and two Fairfax County parks. These parks include George Washington Memorial Parkway (GWMP), Manassas National Battlefield Park (MANA), National Capital Parks – East (NACE), Prince William Forest Park (PRWI), Wolf Trap National Park for the Performing Arts (WOTR), Chesapeake and Ohio National Historical Park (CHOH), Riverbend, and Scott's Run. A brief description of each park is given below and maps are shown in figures 10- 21.

2.1.1 George Washington Memorial Parkway

The GWMP preserves approximately 3,157 ha (7,800 acres) along the Potomac River in Virginia, Maryland and the District of Columbia, from the Piedmont to the Coastal Plain. Natural features of the parkway include deciduous woodlands, rock outcroppings, seasonal pools, alluvial floodplain areas, springs and seepage habitats, streams and freshwater tidal areas. The distinctive topography, drainage patterns and geology along this stretch of the Potomac River provide a great diversity of habitats. First and second order streams in this area are typically fed by groundwater seepages and springs, which in turn flow directly into the Potomac River directly or by way of its tributaries.

2.1.2 Manassas National Battlefield Park

MANA is located within the Triassic basin of the northern Virginia piedmont. The park is characterized by gently rolling hills with a patchwork of open fields and forests. The open habitats generally consist of fescus (*Festuca spp*.), orchard grass (*Dactylis glomerata*) or warm season grass meadows. Forests are predominately either Virginia pine (*Pinus virginiana*)-red cedar (*Juniperus virginiana)*, or basic oak (*Quercus spp*.)-hickory (*Carya spp*.) with some bottomland hardwood forests along the streams.

2.1.3 National Capital Parks – East

NACE includes 14 major park areas and a total of 98 locations within the District of Columbia and three nearby counties in Maryland. The Potomac and Anacostia Rivers dominate the water resources of the park. These two rivers are fed by numerous smaller streams throughout NACE, including Fort Dupont Stream, Watts Branch, Oxon Run, Deep Creek, Still Creek, Accokeek Creek, and Piscataway Creek. Additionally, in Piscataway Park alone, at least 14 unnamed streams drain into the Potomac River. Seeps occur in most of the parks of NACE. In Piscataway and Fort Washington Parks, clay lenses layered between the geologic formations along the base and face of the Tertiary slopelands influence the surface water patterns and account for seasonal seeps. These seeps spring from exposed clay banks in meadows or shallow soil in woods and seasonally discharge because of the changing water table depths.

2.1.4 Prince William Forest Park

PRWI preserves the largest expanse of Piedmont forest in the National Park Service, and includes approximately 70% of the Quantico Creek watershed. This watershed consists of two second order streams, numerous first order tributaries, seeps, vernal pools, springs and wetlands. At 6,475 ha (16,000 acres), the park represents one of the largest parcels of undeveloped land in the area and is the third largest unit of the national park system in Virginia. The park also contains two physiographic provinces, the Piedmont and the Coastal Plain, and it straddles the southern and northern climates, a transition zone that supports many species to the outer limits of their range. About one-fourth of the park lies in the Coastal Plain Physiographic Province and is characterized by stratified marine sediments of sand, silt, clay, and gravel. There are two types of potential amphipod habitat with the Quantico Creek Watershed, seeps and the underflows of the tributaries and creeks.

2.1.5 Wolf Trap National Park for the Performing Arts

WOTR is a 53 ha (130 acre) park located in northern Virginia. Within the landscape at the park are two key streams, Court House Branch Spring and Wolf Trap Run, which are critical natural resources for wildlife and aquatic biota. Additionally the park has known springs, wetlands, and a small pond.

2.1.6 Chesapeake and Ohio Canal National Historical Park from DC to Great Falls

CHOH includes nearly all of the Potomac River floodplain along the Maryland side of the river from Georgetown, D.C. to Cumberland, Maryland. Most of the area is floodplain forest, with some upland forest tracts and areas of agricultural lease use or other altered lands. The park preserves a significant example of eastern deciduous floodplain forest, some of the best remaining and unfragmented tracts of upland deciduous forest in the region (e.g. the Gold Mine Tract at Great Falls), and nationally rare natural communities (e.g. shale barrens in Allegheny District, limestone communities in Piedmont District, and bedrock terrace forests and scoured rock communities from Great Falls to Chain Bridge in Palisades District). River islands in the park provide undisturbed sanctuaries for many plants and animals. The Potomac Gorge, with 400 occurrences of 200 rare species, contains some of the most significant natural areas in Maryland and is one of the more important areas of biodiversity in the National Park Service.

2.1.7 Riverbend Park

Riverbend Park is managed by the Fairfax County Park Authority and encompasses approximately 162 hectares (400 acres) adjacent to the Potomac River north of Great Falls National Park in Fairfax Co. Virginia. Riverbend Park lies in the Piedmont ecoregion, overlying 469 m.y. old schist of the Mather Gorge formation. The park consists primarily of deciduous upland and river floodplain in the Pond Run watershed. 11 distinct vegetative communities, one of which is globally rare, can be found in the park. Riverbend Park also contains permanent and intermittent streams, springs, vernal pools, seeps, and a pond created by an abandoned meander of the Potomac River (Fleming, 2004; Smith).

2.1.8 Scott’s Run Nature Preserve

Scott’s Run Nature Preserve, also managed by the Fairfax County Park Authority, sits adjacent to the Potomac River between Great Falls National Park and the George Washington Memorial Parkway and is primarily within the Scott’s Run watershed. Scott’s Run Nature Preserve encompasses approximately 138 hectares (340 acres). The bedrock of this park is primarily 469 m.y. old phyllite of the Mather Gorge formation. 12 deciduous upland and riparian vegetative communities (one of which is globally rare) can be found in Scott's Run Nature Conserve (Flemming, 2004).

2.2 HYPOTELMINORHEIC SAMPLING

Between February 22, 2006 and May 20, 2007, parks were searched for previously undocumented or unsampled seeps. At putative seeps, visual searches for seep fauna were conducted for at least 30 person minutes. Visual searches were performed by carefully lifting and searching under leaf litter and rocks or disturbing the benthos and allowing macroinvertebrates to wash into small aquarium nets. At some locations, visual searches were complemented by using baited traps to collect seep fauna. Traps were modeled after crayfish traps, and were constructed using plastic water bottles. The tops of the bottles were cut off and inverted into the base with a small piece of shrimp inside as bait. Duct tape was then used to seal the trap which was submerged in the seep and left between 24 and 72 hours (Fig. 6). A YSI© 556 Multi Probe System was used to measure physical and chemical characteristics of hyporheic water. Variables include temperature, pH, dissolved oxygen, salinity, and conductivity (Fig. 7).

Figure 6: Trap used for sampling seeps.

Figure 7: YSI probe used to measure water chemistry.

Nitrate and nitrite levels were measured using a Hach© kit. At some locations, a rotary peristaltic pump was used to collect water for chemical analysis (Fig. 8). A peristaltic pump is useful because it draws water without increasing the dissolved oxygen content of the sample. This is accomplished by submerging the end of a plastic tube attached to the pump. The pump uses rollers on a rotor to create a vacuum within the tube, and as the rotor is turned, water is pushed through the pump into a container until a sufficient volume of water has been collected.

Animals collected from seeps were stored in 70% or 100% ethanol. In the lab, all animals were identified to species when possible. *Stygobromus* species were identified using the key given by Holsinger (1978), *Crangonyx* species were identified using the key given by Zhang and Holsinger (2003), *Gammarus* species were identified using the key given by Holsinger (1972), and *Caecidotea* species were identified using the key given by Williams (1972). Representative amphipod specimens were sent to John R. Holsinger at Old Dominion University for confirmation. Collections are housed in the laboratory of Dr. David C. Culver in the Biology Dept. of American University, 4400 Massachusetts Ave NW, Washington, DC 20016.

Figure 8: Peristaltic pump.

2.3 HYPORHEIC SAMPLING

Invertebrate samples were collected from the hyporheic zone of gravel bars in small streams between May 2, 2006 and January 20, 2007. Potential sampling locations were identified in advance on topographic maps. A Bou-Rouch pump (Fig. 9) was used to extract hyporheic water and invertebrates from a depth of 0 to 1 meter below the water table. The Bou-Rouch pump consists of a hollow steel pipe with holes in one end which is driven into a gravel bar until the holes are below the surface of the groundwater table. A hand pump is attached to the top of this pipe. The system is primed by pouring water into the top of the pump until pumping begins to withdraw groundwater which is collected in a 5 gallon bucket. Gravel bars where sufficient depth could not be obtained, or water could not be extracted (i.e. too much clay or sediment) were not sampled. Because the distribution of interstitial fauna in the hyporheic zone can vary at scales of a few meters within a single gravel bar (Rouch and Lescher-Moutoue, 1992) multiple samples were taken from a single gravel bar in some locations. A YSI 556 Multi Probe System was used to measure physical and chemical characteristics of hyporheic water including temperature, pH, dissolved oxygen, salinity, and conductivity.

Between one and two gallons of hyporheic water were filtered through a fine mesh plankton net to concentrate samples which were subsequently preserved in formalin. In the lab, samples were fully sorted under a dissecting microscope. Amphipods were identified to species based on the key given by Pennak (1989).

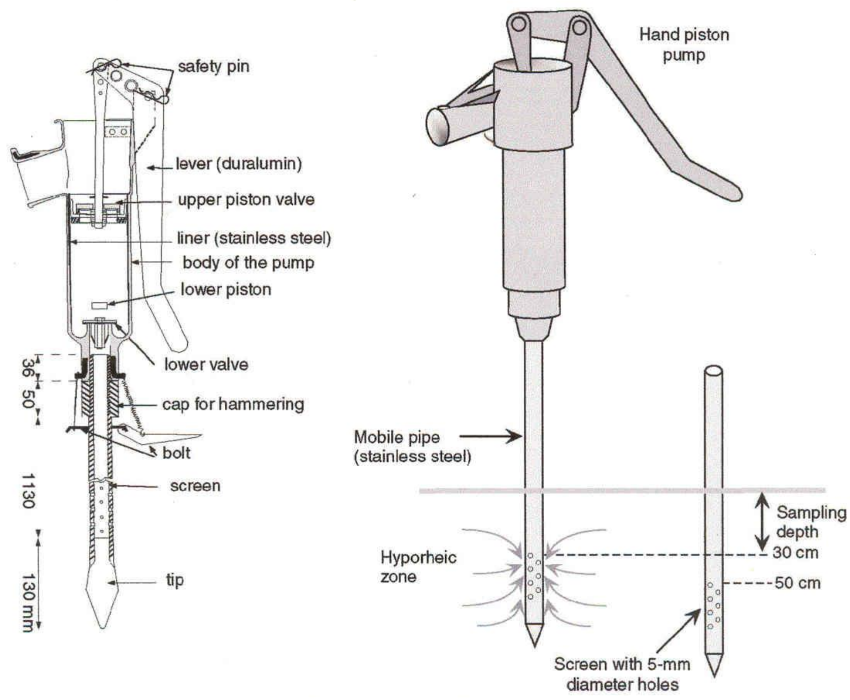

Figure 9: The Bou-Rouch Pump (Malard, 2003)

2.4 GEOGRAPHIC INFORMATION COLLECTION AND REPRESENTATION

For hypotelminorheic and hyporheic sites, GIS coordinates were collected using various Trimble GPS units provided by the Park Service. Between 30 and 60 data points were collected for each site. We attempted to collect data points with PDOP values of six or higher although this was not always feasible because of poor satellite reception. A GPS unit was not used at Wolf Trap, Riverbend, or Scott's Run. Instead, site locations were estimating using field sketches and TOPO! 2.0 (Wildflower Productions). Maps showing site locations were creating using ArcGIS (ESRI) or TOPO! 2.0 and were projected using the NAD 1983 datum.

2.5 DATA ANALYSIS

A Pearson's χ^2 test was performed in SPSS v12.0 to test for an association between the occurrence of *Stygobromus* species and *Caecidotea kenki*. This analysis tested the null hypothesis that seeps were equally likely to harbor *Stygobromus* species only, *Caecidotea kenki* only, both species together, or neither species.

Analyses were also performed to test for differences in the physicochemical characteristics of seeps and hyporheic sites, seeps with and without stygobionts, and seeps with and without *Stygobromus* species. Student's t-tests were performed using SYSTAT v9.0 to test for significant differences in the following seven physicochemical characteristics: nitrate (ppm), nitrite (ppm), temperature (°C), pH, dissolved oxygen (mg/L), salinity (ppm), and conductivity ($\mu s/cm^c$). T-tests were corrected using a Bonferroni correction to reduce the chance of type I error.

SYSTAT v9.0 was used to perform a discriminant analysis and construct an equation for predicting the presence or absence of stygobionts in seeps based on the six physicochemical parameters listed above. Salinity and conductivity are significantly correlated, violating an assumption of discriminant analysis. Consequently, salinity is excluded from this analysis. A backwards stepwise estimation was employed to sequentially exclude physicochemical variables that did not substantially aid in discrimination between seeps with and without stygobionts. Discriminant analysis was also performed for seeps with and without *Stygobromus* species, however, a backwards stepwise estimation could not be used for this analysis.

SPSS v12.0 was used to construct box and whisker plots for each of the seven physicochemical characteristics measured. Box and whisker plots visually describe data by depicting the average value for a dataset, quartiles (partitioning the ordered data into quarters), and outliers (values which are unusually high or low relative to the rest of the data). The graphs were created to describe the data for all hyporheic sites, all seeps, seeps with and without stygobionts, and seeps with and without *Stygobromus* species.

RESULTS

3.1 SAMPLING

Between February of 2006 and May of 2007, 72 putative seeps were sampled for fauna and physicochemical parameters. In addition, 16 hyporheic sites were identified and sampled for fauna and physicochemical parameters. Table two summarizes the sampling dates and number of both hypotelminorheic and hyporheic sites sampled for each of the eight parks sampled.

Table 2: Number of hypotelminorheic and hyporheic sites sampled and sampling dates by park.

Park	Dates sampled	N hypotelminorheic	N hyporheic
GWMP	March 27, 2007 - April 3, 2007	17	2
MANA	February 22, 2007	6	3
NACE	May 2, 2006 - April 24, 2007	10	2
PRWI	February 1, 2007 - May 20, 2007	9	4
WOTR	March 3, 2007	4	2
CHOH	March 24, 2006 - January 21, 2007	7	3
Riverbend	February 3, 2007 - March 18, 2007	10	0
Scott's Run	February 10, 2007 - March 13, 2007	9	0

In addition to these sites, 53 seeps in the George Washington Memorial Parkway that were identified and sampled in 2003-05 (Culver and Chestnut, 2006) are included in this report. Physicochemical data for 21 of the sites from Culver and Chestnut were also included in statistical analyses. Sites that were used for statistical analyses of physicochemical data are identified in appendix I. Figures 10-21 illustrate the location of seeps in each park, and appendix I lists GPS coordinates for each site. Appendix II lists GPS coordinates for hyporheic sites.

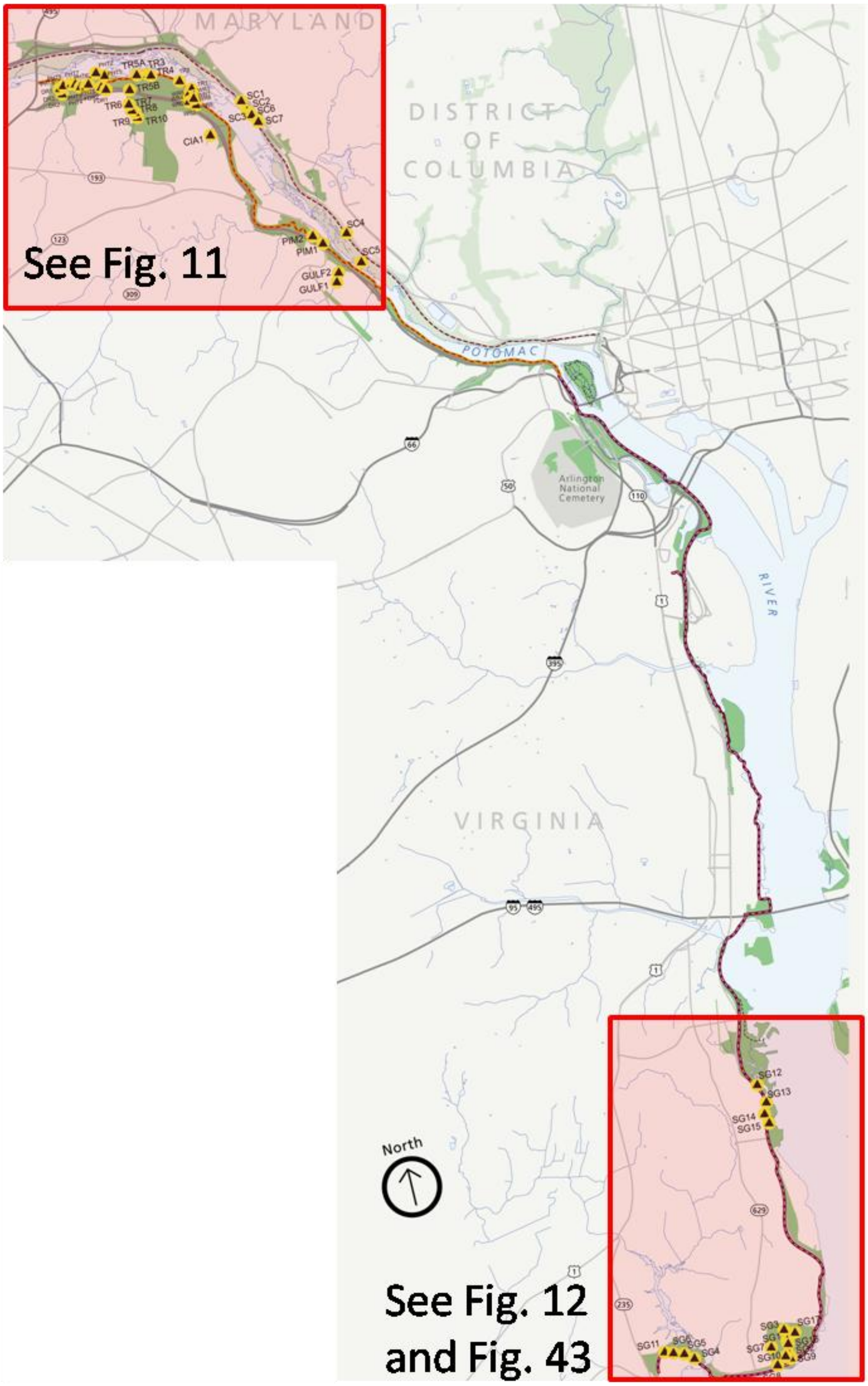

Figure 10: Seeps sampled in George Washington Memorial Parkway and location of smaller scale maps. Map by Tammy Stidham and Ben Hutchins.

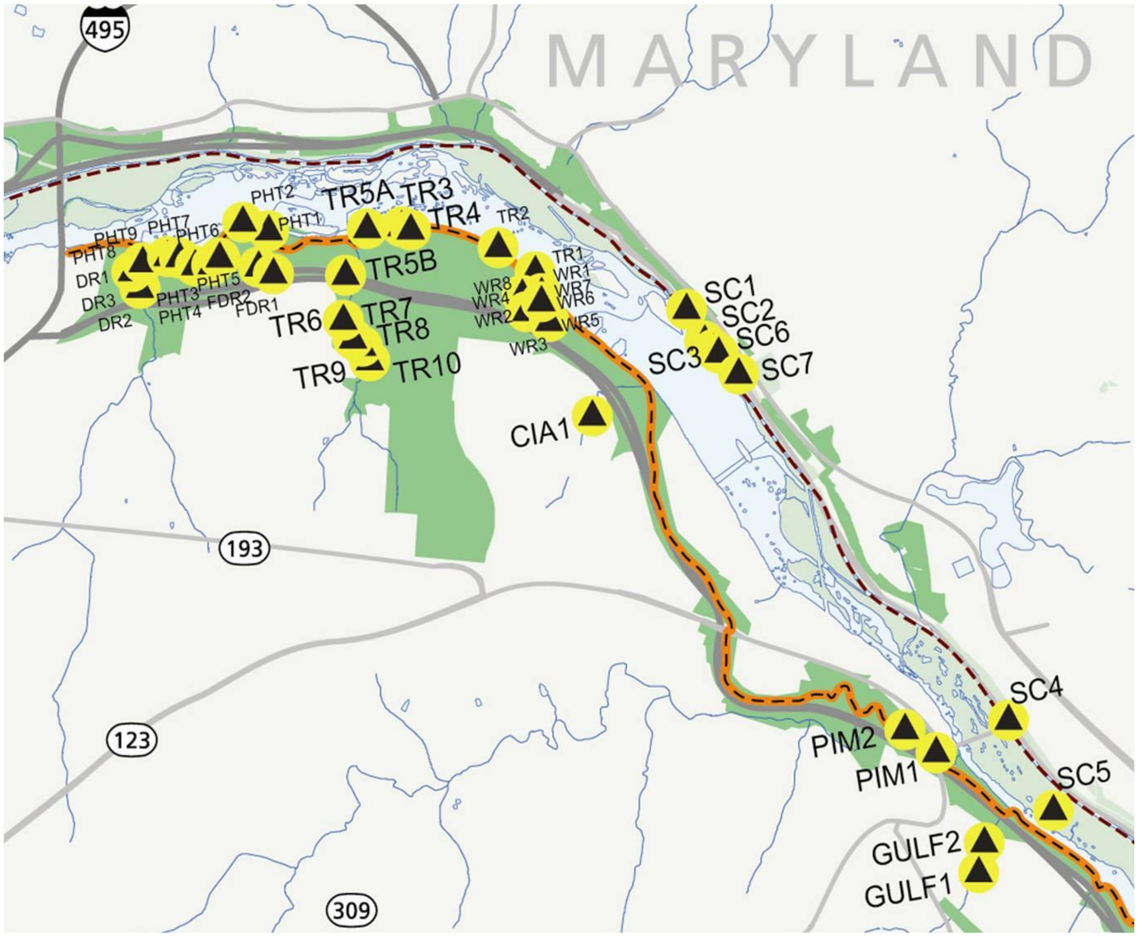

Figure 11: Seeps sampled in northern George Washington Memorial Parkway (see Fig. 10 for relative location). Map by Tammy Stidham.

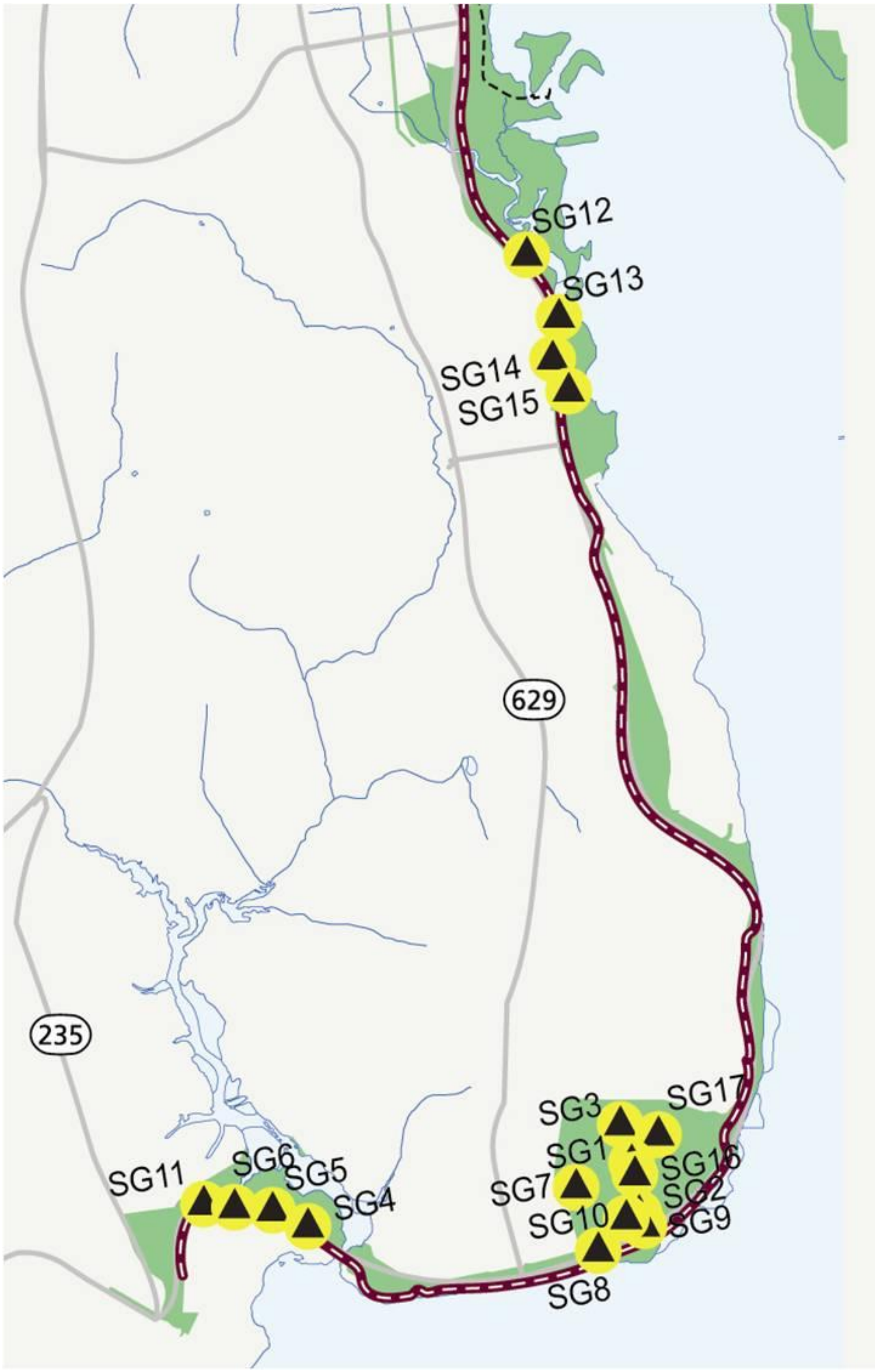

Figure 12: Seeps sampled in southern George Washington Memorial Parkway (see Fig. 10 for relative location). Map by Tammy Stidham.

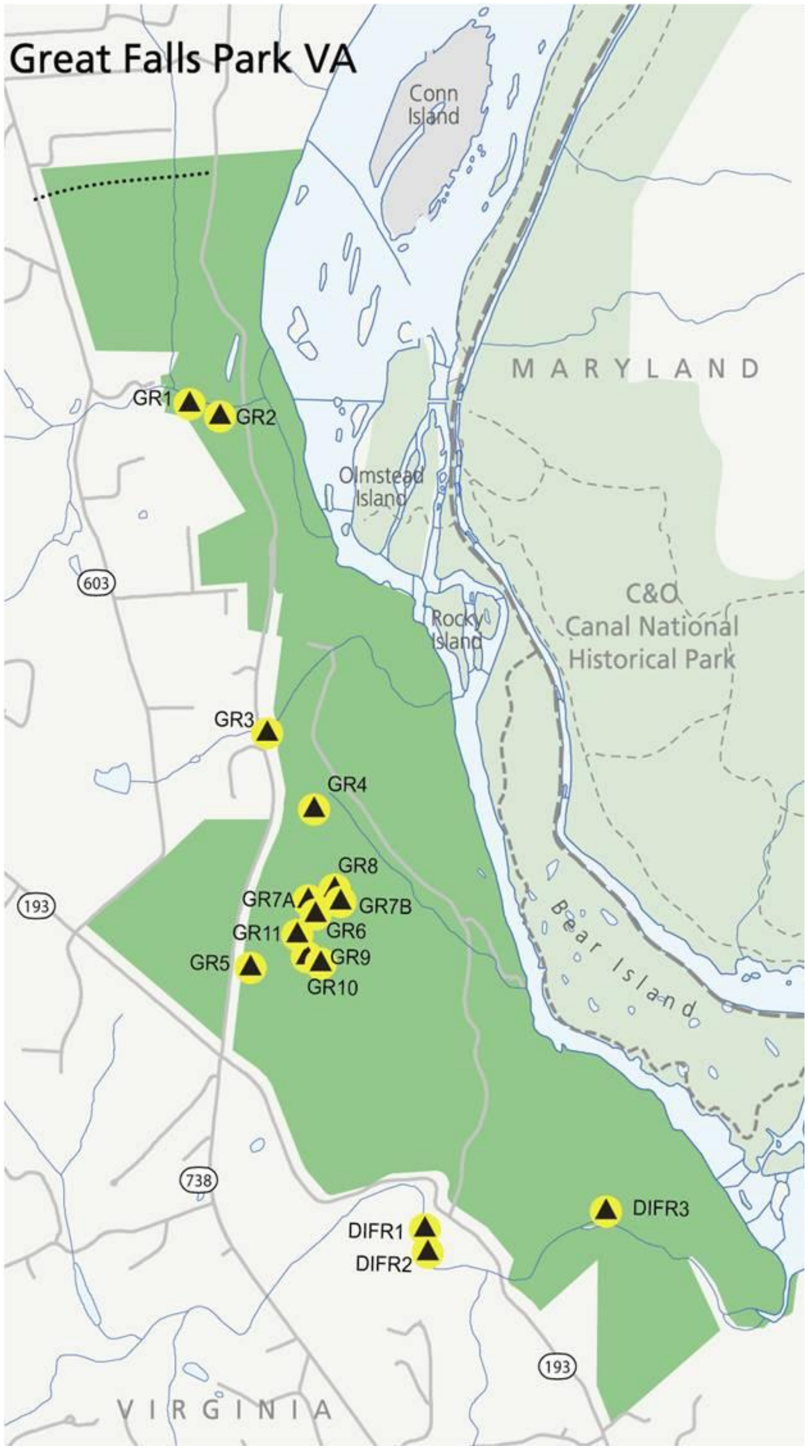

Figure 13: Seeps sampled in and near Great Falls Park. Map by Tammy Stidham.

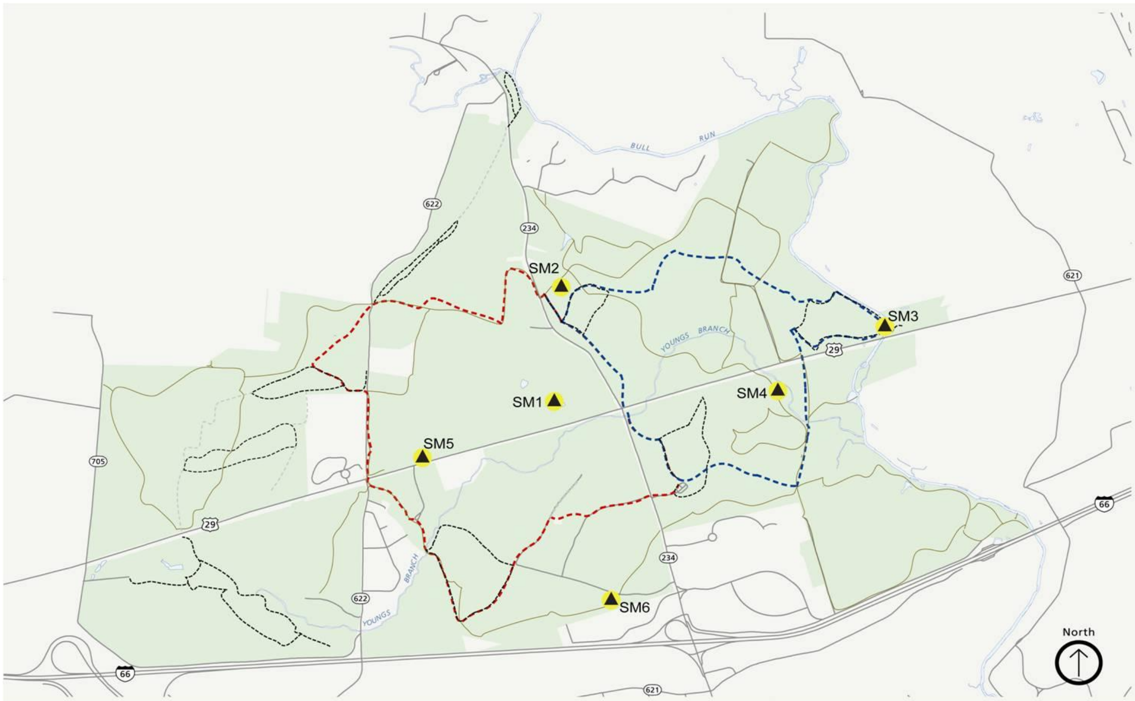

Figure 14: Seeps sampled in Manassas National Battlefield Park. Map by Tammy Stidham.

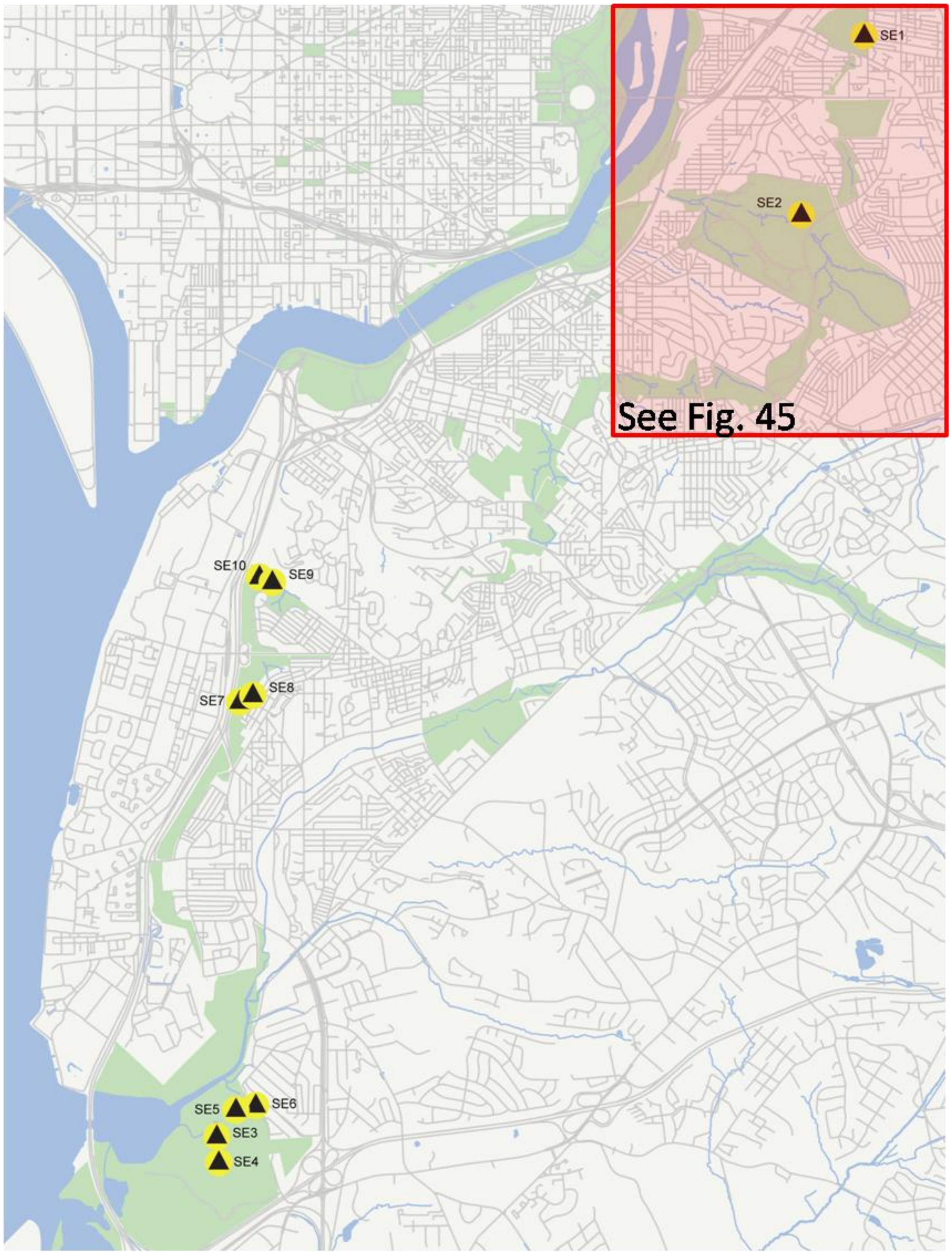

Figure 15: Seeps sampled in National Capital Parks - East and location of smaller scale map. Map by Tammy Stidham and Ben Hutchins.

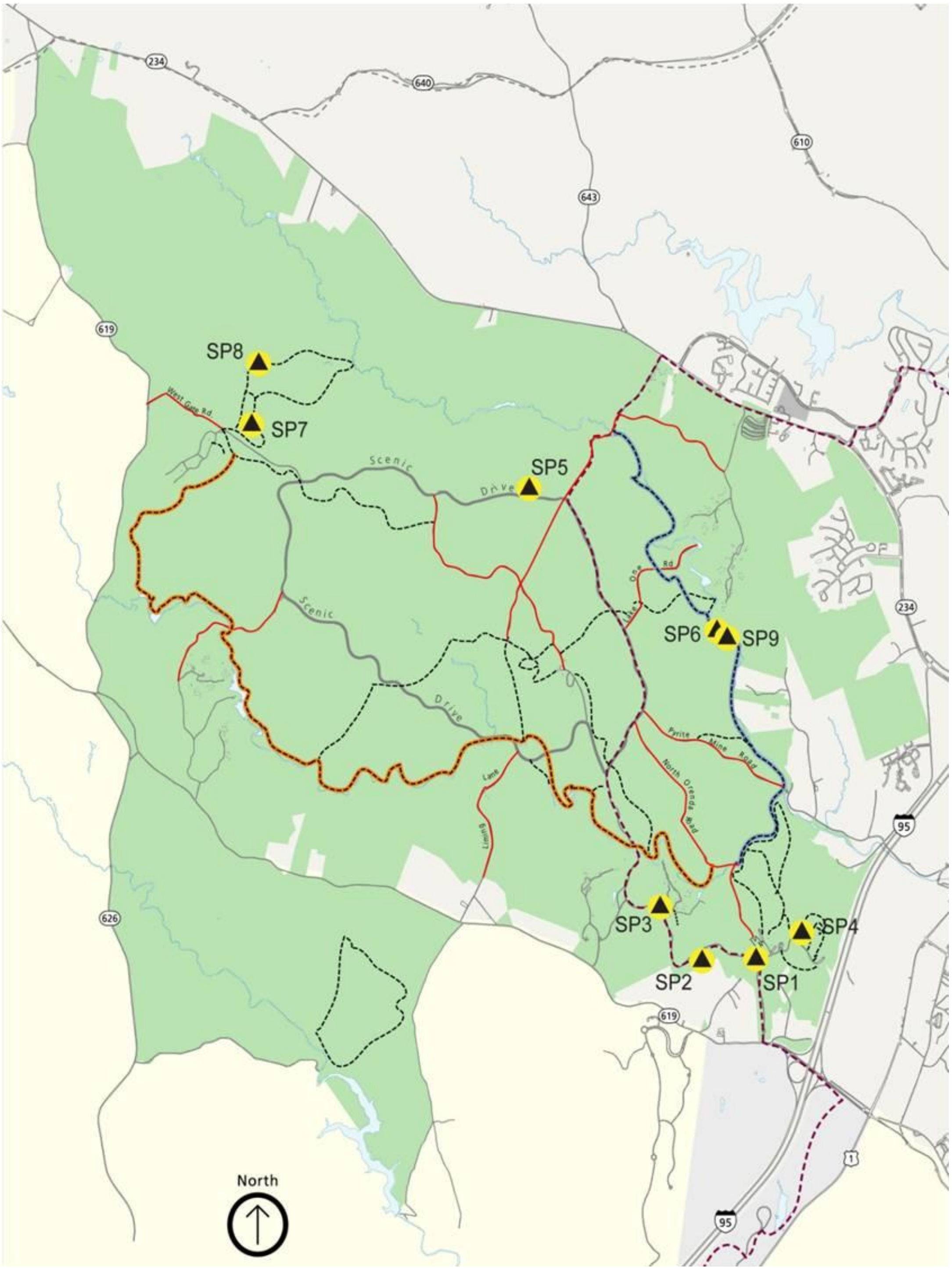

Figure 16: Seeps sampled in Prince William Forest Park. Map by Tammy Stidham.

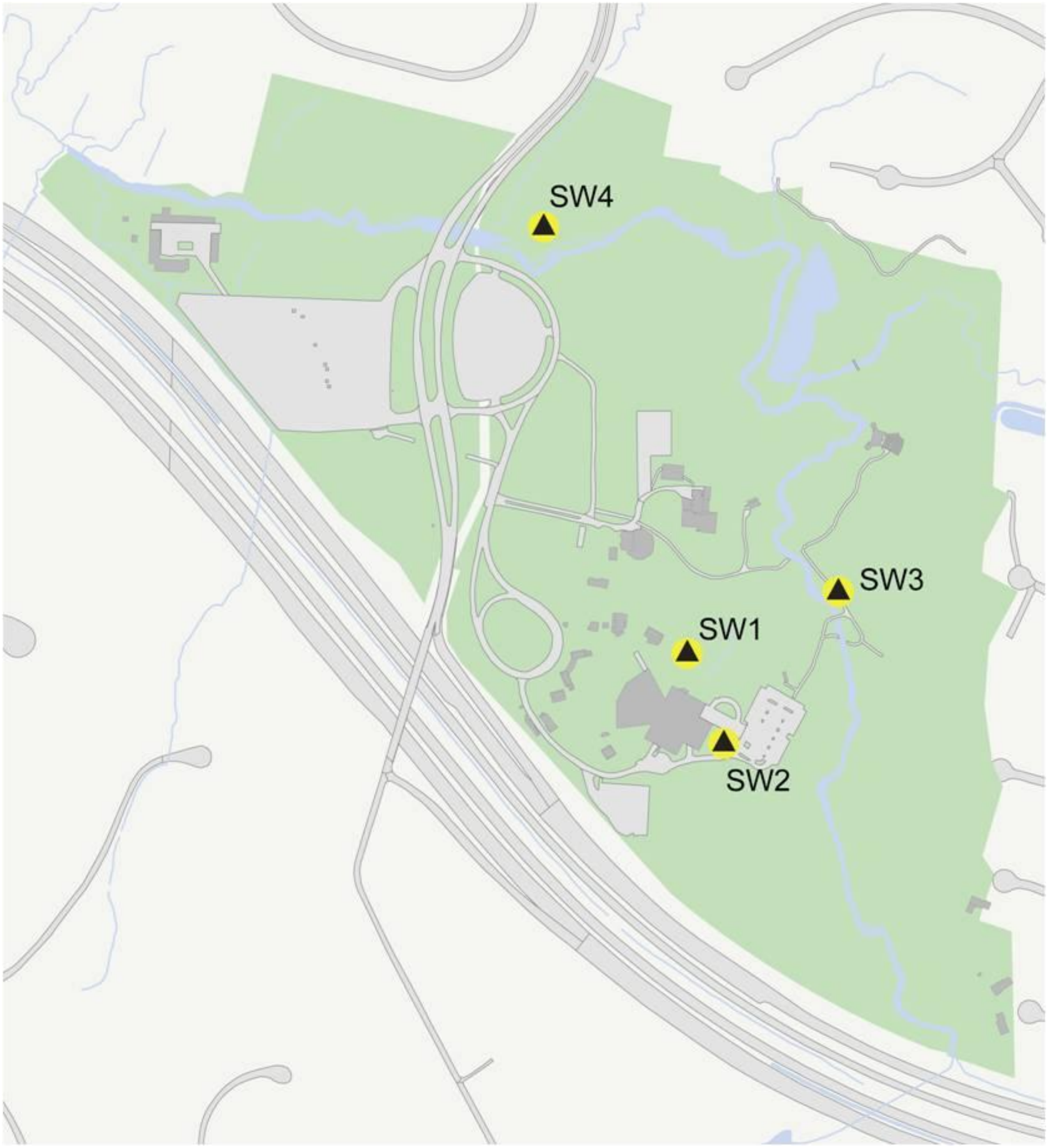

Figure 17: Seeps sampled in Wolf Trap National Park for the Performing Arts. Map by Tammy Stidham.

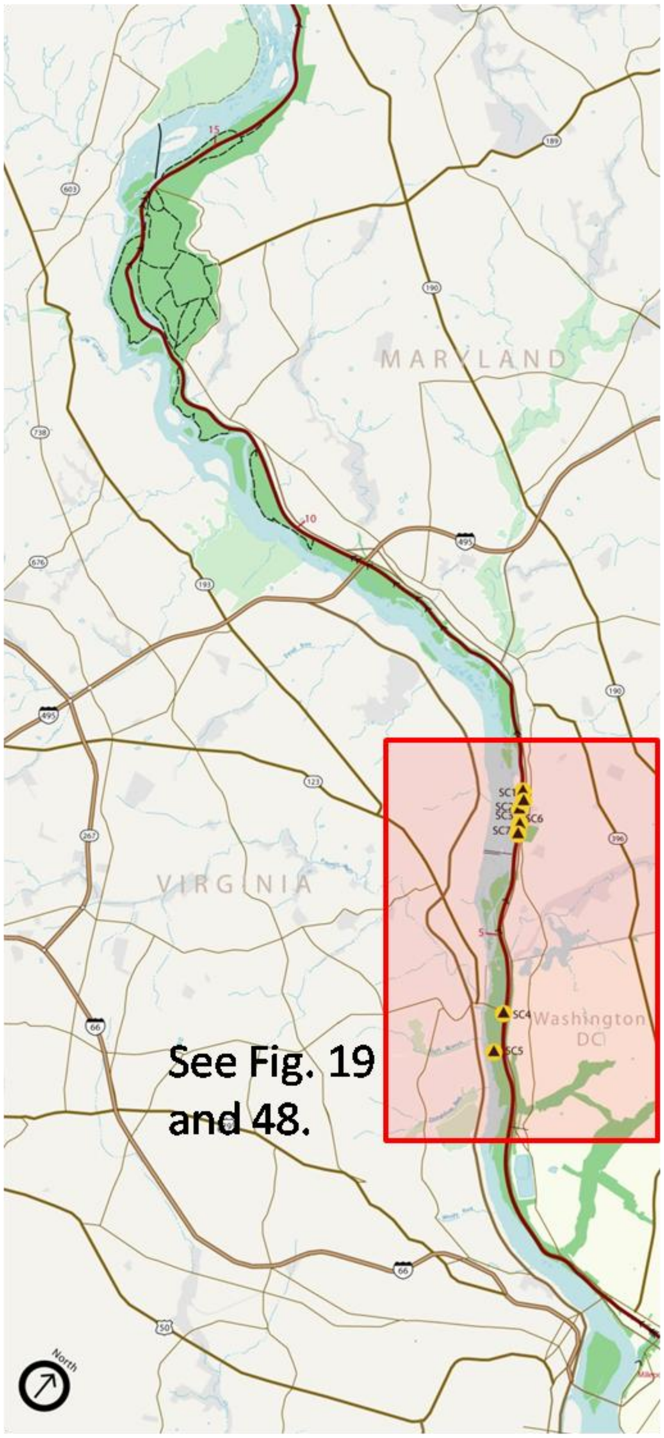

Figure 18: Seeps sampled in Chesapeake and Ohio Canal National Historical Park and location of smaller scale map. Map by Tammy Stidham and Ben Hutchins.

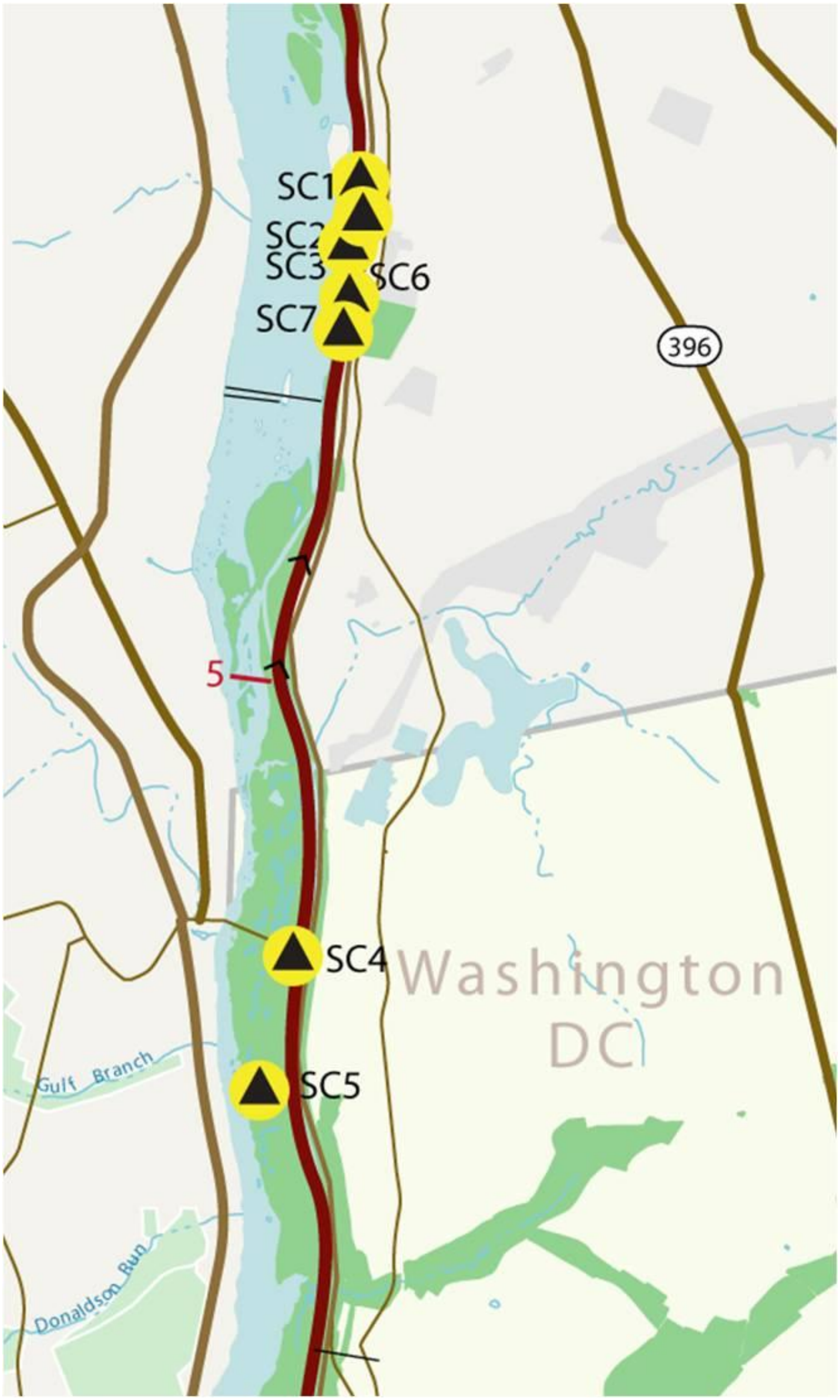

Figure 19: Small scale map of seeps sampled in Chesapeake and Ohio Canal National Historical Park. Map by Tammy Stidham.

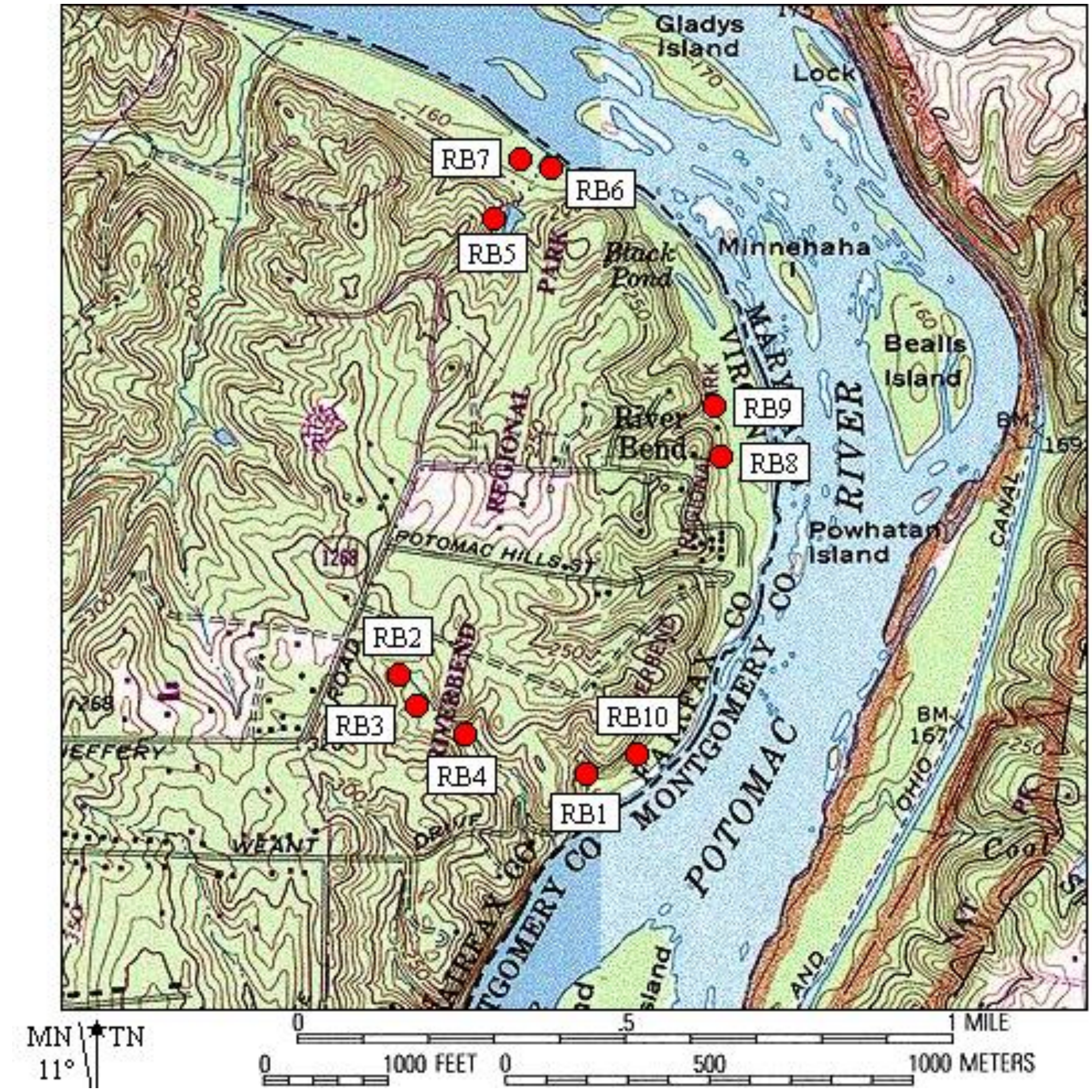

Figure 20: Seeps sampled in Riverbend Park. Map by Ben Hutchins.

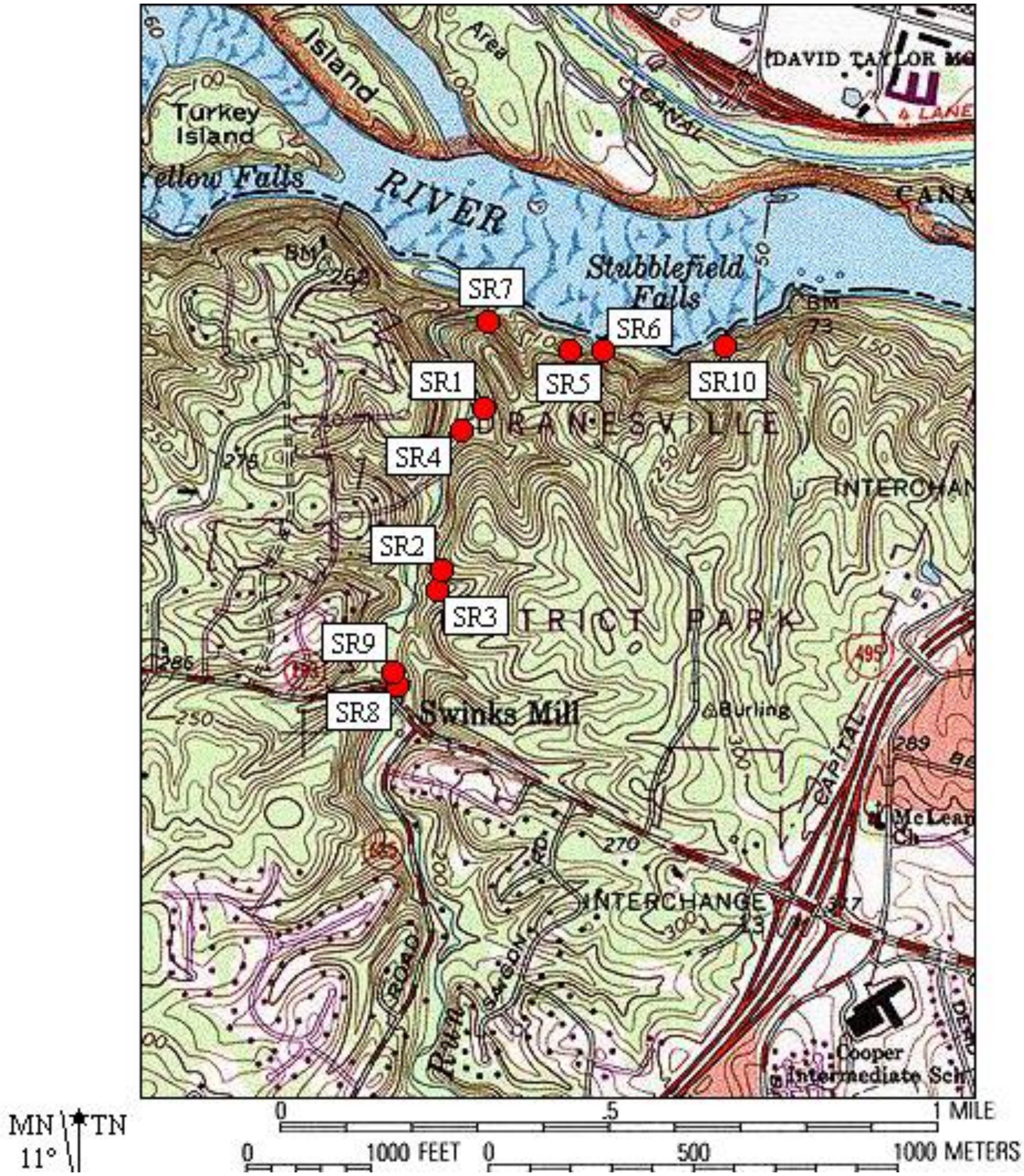

Figure 21: Seeps sampled in Scott's Run Park. Map by Ben Hutchins

3.2 SPECIES OCCURRENCE

Eight species of amphipod, three species of isopod, and one species of snail were identified from seeps during this study. Of these, three species of amphipod (*Stygobromus tenuis potomacus*, *S. pizzinii*, and *S. sextarius*), the isopod *Caecidotea kenki*, and the snail *Fontigens bottimeri* are considered obligate inhabitants of seeps and are considered stygobionts. Of the 126 seeps sampled, 62 sites (49.2%) were found to have one or more species of stygobiont, and 49 sites (38.9%) were found to have one or more species of *Stygobromus.* No stygobionts were identified from any of the 15 hyporheic sites sampled. Faunal samples from hyporheic sites were typically dominated by copepods of the order *Cyclopoida* or benthic insects. Two amphipods were collected from hyporheic sites. *Crangonyx shoemakeri* was collected from HC1 in CHOH (appendix II) and *Synurella chamberlaini* was collected from HG2 in GWMP (appendix II). Table three lists the seeps at which amphipods were collected and table four lists seeps at which isopods and the snail *F. bottimeri* were collected. Table five illustrates the number of seeps sampled at each park and the number of sites containing stygobionts, *Stygobromus, Caecidotea kenki,* and *Fontigens bottimeri*. A Pearson's χ^2 test was run to test for a relationship between the occurrence of *Stygobromus* species and *Caecidotea kenki.* Table six illustrates that both *Stygobromus* species and *Caecidotea kenki* occur alone less frequently than expected. Furthermore, sites contain both species together more frequently than expected given no association between the two species. Given these data, the null hypothesis of no association between the occurrence of *Stygobromus* species and *Caecidotea kenki* was rejected $\chi2 = 7.216$, $p = 0.007$.

Table 3: Amphipods identified from seep sites. GW are GWMP sites that were sampled during 2003-05. * denotes stygobionts. Sites highlighted in yellow contain one or more species of stygobiont.**

Park	Site name	*Stygobromus tenuis potomacus**	*S. pizzinii**	*S. sextarius**	*S. sp.***	*Crangonyx shoemakeri*	*C. stagnicolous*	*C. palustris*	*C. sp.*	*Gammarus minus*	*G. fasciatus*	*G. sp.*
GWMP	SG1					X						
	SG2	X										
	SG3											
	SG4								X			
	SG5											
	SG6						X					
	SG7	X										
	SG8	X										
	SG9	X										
	SG10	X										
	SG11						X					
	SG12	X				X						
	SG13	X										
	SG14	X										
	SG15	X										
	SG16							X				
	SG17											
GW**	FDR1											
	FDR2											
	PHT1											
	PHT2					X						
	PHT3											
	PHT4											
	PHT5									X		
	PHT6											
	PHT7											
	PHT8		X									
	PHT9	X										
	DR1											
	DR2											X
	DR3	X	X									
	WR1	X	X									
	WR2											
	WR3		X									
	WR4					X						
	WR5		X									
	WR6											
	WR7											
	TR1					X						
	TR2	X	X	X		X				X		
	TR3											X
	TR4					X						
	TR5A											X
	TR5B											
	TR6											
	TR7									X		
	TR8											
	TR9											
	TR10											

Table 3 continued.

Park	Site name	*Stygobromus tenuis potomacus**	*S. pizzinii**	*S. sextarius**	*S. sp.**	*Crangonyx shoemakeri*	*C. stagnicolous*	*C. palustris*	*C. sp.*	*Gammarus minus*	*G. fasciatus*	*G. sp.*
GW**	PIM1	X	X	X								
cont.	PIM2	X	X									
	GULF1	X	X									
	GULF2	X	X									
	CIA1				X							
	DIFR1	X				X						
	DIFR2	X				X						
	DIFR3											
	WR8											
	GR1	X				X						
	GR2					X						
	GR3					X					X	
	GR4											
	GR8				X							
	GR7B											
	GR7A	X				X						
	GR6											
	GR9											
	GR10	X				X						
	GR11					X						
	GR5			X								
MANA	SM1	X							X			
	SM2	X				X						
	SM3											
	SM4					X						
	SM5	X										
	SM6											
NACE	SN1	X										
	SN2	X										
	SN3					X						
	SN4	X										
	SN5											
	SN6	X										
	SN7	X										
	SN8	X										
	SN9											
	SN10											
PRWI	SP1											
	SP2					X						
	SP3					X						
	SP4					X						
	SP5	X										
	SP6			X		X						
	SP7											
	SP8											
	SP9	X				X						

Table 3 continued.

Park	Site name	*Stygobromus tenuis potomacus**	*S. pizzinii**	*S. sextarius**	*S. sp.**	*Crangonyx shoemakeri*	*C. stagnicolous*	*C. palustris*	*C. sp.*	*Gammarus minus*	*G. fasciatus*	*G. sp.*
WOTR	SW1					X						
	SW2	X				X						
	SW3	X				X						
	SW4	X										
CHOH	SC1		X									
	SC2					X						
	SC3		X			X						
	SC4					X				X		
	SC5								X			
	SC6		X			X						
	SC7		X			X						
RVBD	RB1					X						
	RB2					X						
	RB3					X						
	RB4		k			X						
	RB5					X						
	RB6											
	RB7											
	RB8					X						
	RB9											
	RB10											
SCRN	SR1	X				X						
	SR2					X						
	SR3					X						
	SR4	X				X						
	SR5											
	SR6					X					X	
	SR7											
	SR8											
	SR9											
	SR10											

Table 4: Isopods and snails identified from seep sites. GWMP sites were sampled during 2003-05. * denotes stygobionts. Site highlighted in yellow contain one or more stygobionts**

		Isopods				Snails
Park	Site name	*Caecidotea kenki**	*C. nodulus*	*C. forbesi*	*C. sp.*	*Fontigens bottomeri**
GWMP	SG1					
	SG2					
	SG3					
	SG4					
	SG5					
	SG6		X			
	SG7					
	SG8					
	SG9					
	SG10					
	SG11		X			
	SG12		X			
	SG13					
	SG14					
	SG15					
	SG16		X			
	SG17					
GWMP**	FDR1					
	FDR2					
	PHT1					
	PHT2				X	
	PHT3					
	PHT4					
	PHT5					
	PHT6					
	PHT7					
	PHT8					
	PHT9					
	DR1					
	DR2	X				
	DR3					
	WR1	X				
	WR2	X				
	WR3	X				
	WR4	X				
	WR5	X				
	WR6					
	WR7					
	TR1					
	TR2	X				
	TR3	X				
	TR4	X				X
	TR5A	X				
	TR5B					
	TR6					
	TR7					
	TR8					
	TR9				X	
	TR10					

Table 4 continued.

		Isopods				Snails
Park	Site name	*Caecidotea kenki**	*C. nodulus*	*C. forbesi*	*C. sp.*	*Fontigens bottomeri**
GWMP** cont.	PIM1	X				
	PIM2	X				
	GULF1				X	
	GULF2	X				
	CIA1	X				
	DIFR1	X				X
	DIFR2	X				X
	DIFR3					
	WR8					
	GR1					
	GR2				X	
	GR3					
	GR4					
	GR8					
	GR7B					
	GR7A					X
	GR6					
	GR9					X
	GR10				X	
	GR11				X	X
	GR5				X	
MANA	SM1					
	SM2					
	SM3	X				
	SM4					
	SM5					
	SM6					
NACE	SN1					
	SN2					
	SN3			X		
	SN4					
	SN5					
	SN6					
	SN7					
	SN8					
	SN9					
	SN10					
PRWI	SP1					
	SP2					
	SP3					
	SP4	X				
	SP5					
	SP6		X			X
	SP7					
	SP8		X			
	SP9		X			

Table 4 continued.

		Isopods				Snails
Park	Site name	*Caecidotea kenki**	*C. nodulus*	*C. forbesi*	*C. sp.*	*Fontigens bottomeri**
WOTR	SW1					
	SW2	X				
	SW3					
	SW4					
CHOH	SC1				X	
	SC2	X				
	SC3	X				
	SC4				X	
	SC5			X		
	SC6	X				
	SC7	X				
Riverbend	RB1					
	RB2					
	RB3					
	RB4					
	RB5				X	
	RB6					
	RB7					
	RB8					
	RB9					
	RB10					
Scott's Run	SR1	X				X
	SR2	X				
	SR3	X				
	SR4	X				X
	SR5					
	SR6				X	
	SR7					
	SR8					
	SR9					
	SR10					

Table 5: N = number of seeps sampled. The number of sites with and without stygobionts and the number of sites with *Stygobromus*, *Caecidotea kenki*, or *Fontigens bottimeri* are shown.

	N	Sites with Stygobites	Sites Without Stygobites	Sites with Stygobromus sp.	*Stygobromus* and *Caecidotea kenki*	*Stygobromus* and no *Caecidotea kenki*	*Caecidotea kenki* and no *Stygobromus*	*Fontigens bottimeri*
GWMP	17	9	8	9	0	9	0	0
GWMP**	53	25	28	19	10	9	6	6
MANA	6	4	2	3	0	3	1	0
NACE	10	6	4	6	0	6	0	0
PRWI	9	4	5	3	1	2	1	1
WOTR	4	3	1	3	1	2	0	0
CHOH	7	5	2	4	3	1	1	0
Riverbend	10	0	10	0	0	0	0	0
Scott's Run	10	4	6	2	2	0	2	2
Total	109	51	66	49	17	32	11	9

Table 6: Actual number of seeps containing *Stygobromus* species and/or *Caecidotea kenki* and expected values calculated via Pearson's χ^2.

			Caecidotea kenki	
			present	**absent**
Stygobromus	**present**	**count**	17	32
		expected	10.89	38.11
	absent	**count**	11	66
		expected	17.11	59.89

3.3 PHYSICOCHEMICAL CHARACTERISTICS OF SEEPS AND HYPORHEIC SITES

Table seven lists descriptive statistics for the seven physicochemical parameters measured for seep and hyporheic sites: Temperature (°C), pH, Salinity (ppt), Conductivity (µS/cm), Nitrate (ppm) Nitrite (ppm), and DO (mg/L). This data is visually represented by the boxplots in figures 22 through 28 which show mean values, quartiles, and outlying values for these seven parameters.

Table 7: Descriptive statistics for seven physicochemical parameters of seeps and hyporheic sites. Std. Dev. = Standard Variation, Coeff. Var. = Coefficient of Variation.

Parameter	Habitat	N	Mean	Min	Max	Std. Dev.	Std. Error Mean	Coeff. Var.
Temperature °C	seep	90	11.40	0.05	22.50	5.283	0.557	0.463
	hyporheic	15	12.64	2.33	18.88	4.780	1.240	0.379
pH	seep	84	6.395	4.10	9.74	1.129	0.123	0.177
	hyporheic	15	7.54	6.43	9.06	0.680	0.180	0.091
Salinity (ppt)	seep	77	0.10	0.01	0.37	0.090	0.010	0.899
	hyporheic	15	0.14	0.02	0.35	0.100	0.030	0.726
Conductivity (µS/cm)	seep	78	155.55	9.16	673.00	142.910	16.180	0.919
	hyporheic	15	221.80	44.00	534.00	154.270	39.830	0.696
Nitrate (ppm)	seep	65	1.08	0.00	5.00	1.300	0.160	1.208
	hyporheic	8	0.49	0.10	1.50	0.440	0.160	0.907
Nitrite (ppm)	seep	65	0.01	0.00	0.20	0.040	0.010	3.574
	hyporheic	8	0.06	0.01	0.20	0.060	0.020	1.058
DO (mg/L)	seep	90	6.27	0.00	14.06	2.980	0.310	0.476
	hyporheic	11	4.30	1.62	6.23	1.270	0.380	0.295

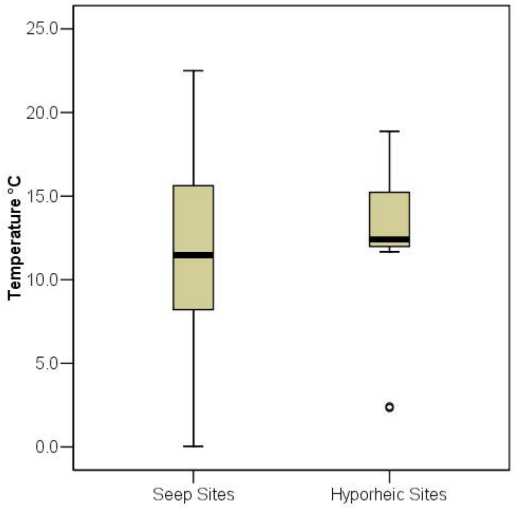

Figure 22: Boxplots depicting mean values, quartiles, and outlying values for temperature of seep and hyporheic sites.

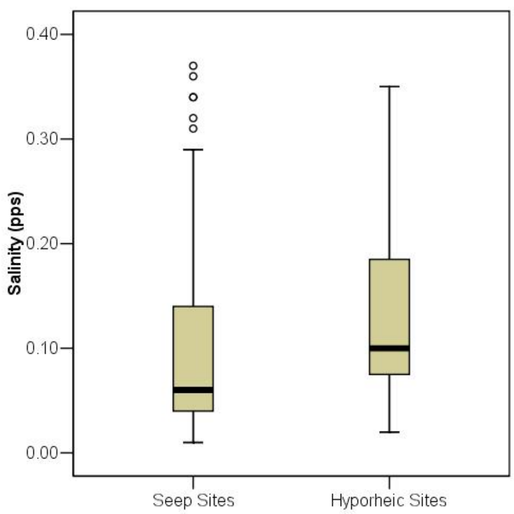

Figure 23: Boxplots depicting mean values, quartiles, and outlying values for salinity of seep and hyporheic sites.

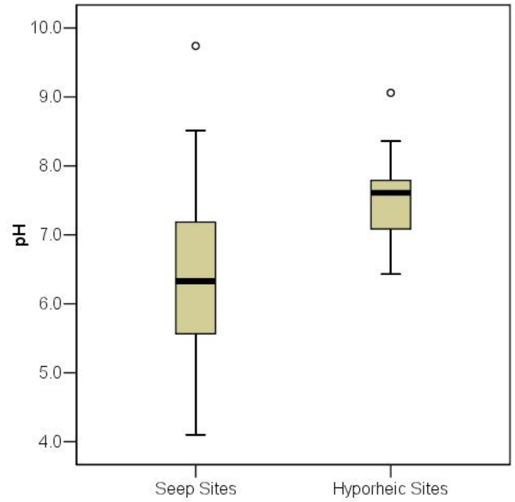

Figure 24: Boxplots depicting mean values, quartiles, and outlying values for pH of seep and hyporheic sites.

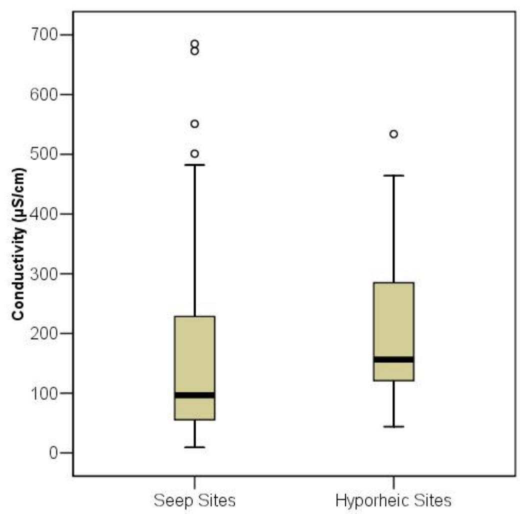

Figure 25: Boxplots depicting mean values, quartiles, and outlying values for conductivity of seep and hyporheic sites.

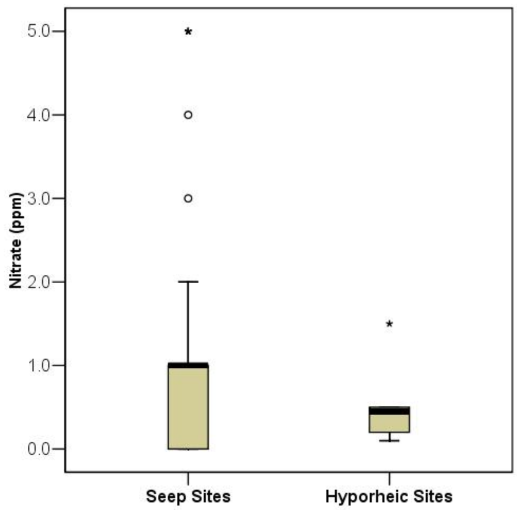

Figure 26: Boxplots depicting mean values, quartiles, outlying values, and extreme values for nitrate of seep and hyporheic sites.

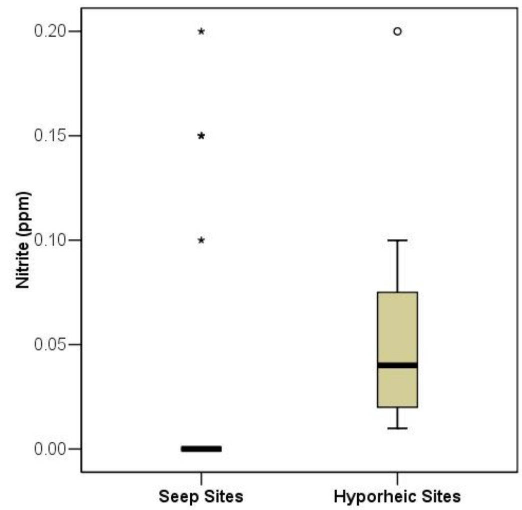

Figure 28: Boxplots depicting mean values, quartiles, outlying values and extreme values for nitrite of seep and hyporheic sites.

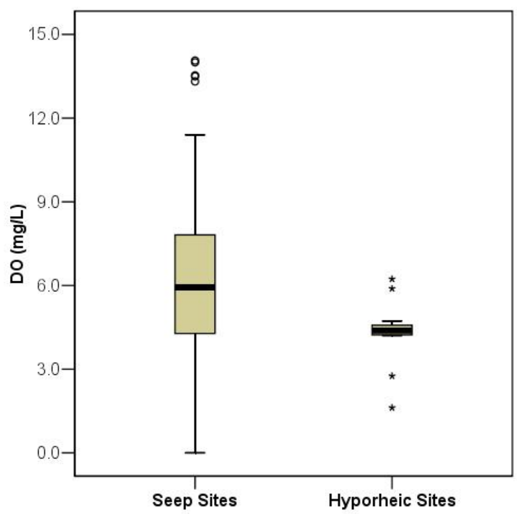

Figure 27: Boxplots depicting mean values, quartiles, outlying values, and extreme values for dissolved oxygen of seep and hyporheic sites.

Table eight depicts the results of seven Student's t-tests which were performed to test for significant differences in the physicochemical parameters of seep and hyporheic sites. These tests revealed significant differences in mean values of pH and dissolved oxygen. Specifically, seeps had a lower mean pH and a higher dissolved oxygen content. For both of these variables, seeps were found to be more variable than hyporheic sites, as indicated by the coefficient of variation (Table 7). Of note, five seeps contained measurable concentrations of nitrite. Three of these DIFR 1, DIFR2, DIFR3, all in Difficult Run in the George Washington Memorial Parkway, were sampled for an earlier study (Culver and Chestnut, 2003). The other two, GWMP14 and WT1, were found in the George Washington Memorial Parkway and Wolf Trap, respectively. *Stygobromus*, or other stygobionts were found in three of these sites: DIFR1, DIFR2, and GWMP14 (Tables 3-4). Other parameters were not significantly different between seeps and hyporheic sites.

Table 8: Results of Student's t-tests comparing mean values for seven physicochemical parameters between seep and hyporheic sites. Mean difference = hyporheic mean – seep mean.

Parameter	t	d.f.	Mean Difference	Corrected Sig. (2-tailed)	Uncorrected Sig. (2-tailed)
Temperature °C	0.913	20.1	1.238	1.000	0.372
pH	5.327	29.8	1.145	0.000	0.000
Salinity (ppt)	1.398	18.6	0.040	1.000	0.179
Conductivity (µS/cm)	1.541	18.9	66.252	1.000	0.140
Nitrate (ppm)	2.633	26.7	-0.592	0.111	0.014
Nitrite (ppm)	2.106	7.7	0.048	0.556	0.069
DO (mg/L)	3.991	26.8	-1.975	0.004	0.000

3.4 PHYSICOCHEMICAL CHARACTERISTICS OF SEEPS WITH AND WITHOUT STYGOBIONTS

Table nine lists descriptives for the seven physicochemical parameters measured for seep sites in which stygobionts were and were not found. This data is visually represented by the boxplots in figures 29 through 35 which show mean values, quartiles, and outlying values for these seven parameters.

Table 9: Descriptives of seven physicochemical parameters for seeps where stygobionts were and were not found. Std. Dev. = standard deviation. Coeff. Var. = coefficient of variation.

Parameter	Stygobiont occurrence	N	Mean	Min	Max	Std. Dev.	Std. Error Mean	Coeff. Var.
Temp °C	Present	55	11.41	1.35	21.53	5.200	0.700	0.456
	Absent	35	11.38	0.05	22.50	5.480	0.930	0.482
pH	Present	52	6.236	4.30	8.51	1.053	0.146	0.169
	Absent	32	6.652	4.10	9.74	1.215	0.215	0.183
Salinity (ppt)	Present	46	0.10	0.01	0.37	0.100	0.010	0.955
	Absent	31	0.10	0.02	0.36	0.080	0.010	0.817
Cond (µS/cm)	Present	45	152.34	10.00	551.00	142.690	21.270	0.937
	Absent	33	159.92	9.16	673.00	145.320	25.300	0.909
Nitrate (ppm)	Present	40	0.98	0.00	5.00	1.340	0.210	1.366
	Absent	25	1.24	0.00	5.00	1.260	0.250	1.016
Nitrite (ppm)	Present	40	0.01	0.00	0.20	0.040	0.010	3.595
	Absent	25	0.01	0.00	0.15	0.040	0.010	3.536
DO (mg/L)	Present	55	6.42	0.00	14.06	3.060	0.410	0.477
	Absent	35	6.04	1.27	13.53	2.880	0.490	0.477

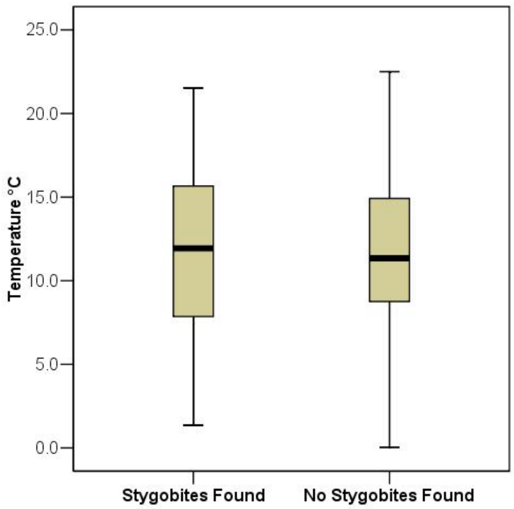

Figure 29: Boxplots depicting mean values and quartiles for temperature of seeps where stygobionts were and were not found.

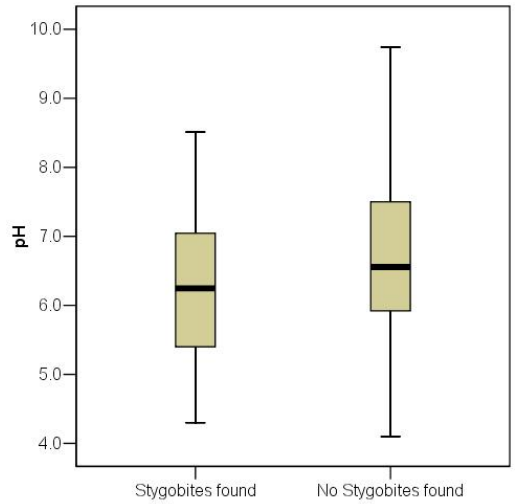

Figure 31: Boxplots depicting mean values, quartiles, and outlying values for pH of seeps where stygobionts were and were not found.

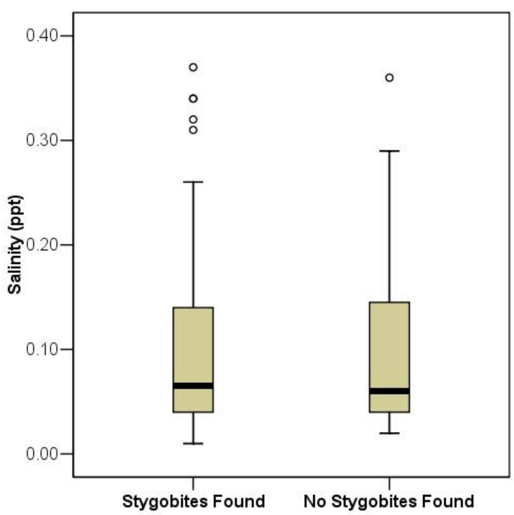

Figure 30: Boxplots depicting mean values, quartiles, and outlying values for salinity of seeps where stygobionts were and were not found.

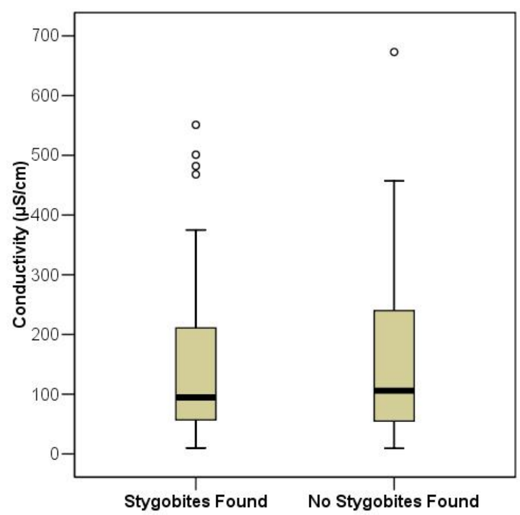

Figure 32: Boxplots depicting mean values, quartiles, and outlying values for conductivity of seeps where stygobionts were and were not found.

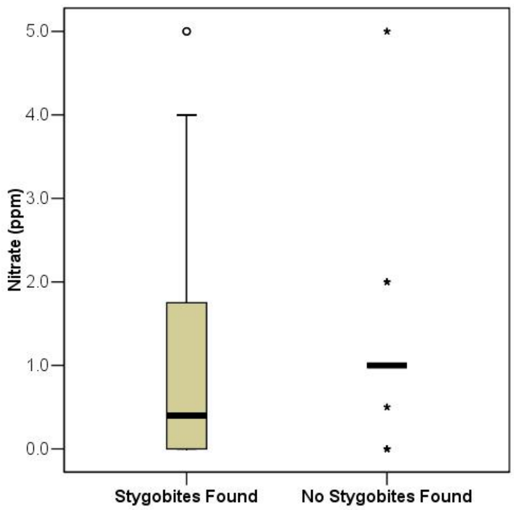

Figure 33: Boxplots depicting mean values, quartiles, and outlying values for conductivity of seeps where stygobionts were and were not found.

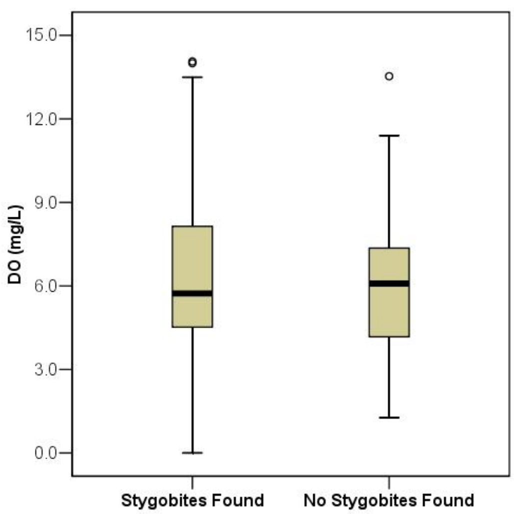

Figure 34: Boxplots depicting mean values, quartiles, and outlying values for conductivity of seeps where stygobionts were and were not found.

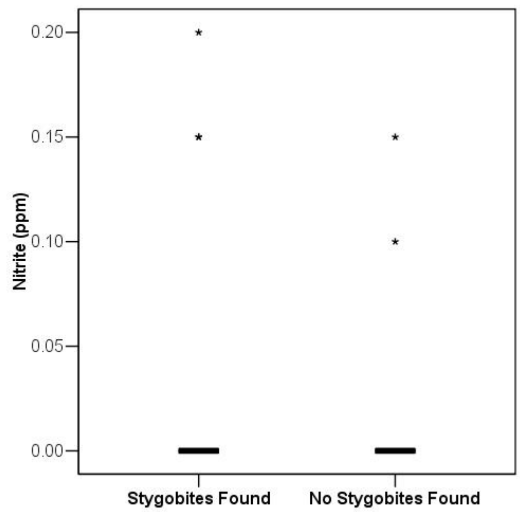

Figure 35: Boxplots depicting mean values, quartiles, and outlying values for conductivity of seeps where stygobionts were and were not found.

Table ten depicts the results of seven Student's t-tests which were performed to test for significant differences in the physicochemical parameters of seeps where stygobionts were and were not found. These tests revealed no significant differences between seeps for all parameters.

Discriminant analysis revealed that no variable or group of variables could be used to effectively discriminate between seeps with and without stygobionts. Equations one and two incorporate all six physicochemical variables into discriminant functions for seeps with and without stygobionts. For these six variables, seeps with and without stygobionts are different only at the $p = 0.864$ level (Wilks' lambda). These variables can be used to successfully predict the absence of stygobionts 60% of the time and correctly predict the presence of stygobionts 69% of the time. When evaluated at the group means for each parameter, equations one and two equaled -0.469 for seeps without stygobionts and 0.323 for seep with stygobionts. This suggests that when evaluated for new seeps, these equations yield positive values for seeps with stygobionts and negative values for seeps without stygobionts. Discriminant analysis using backwards stepwise removal of variables revealed that no single variable or subset of physicochemical variables explained most of the variation between seeps with and without stygobionts. Backwards stepwise exclusion resulted in the exclusion of all variables and could therefore not be used to produce a discriminate function using less than all six variables.

Table 10: Results of Student's t-tests comparing mean values for seven physicochemical parameters between seeps where stygobionts were and were not found. Mean difference = mean for seeps without stygobionts – mean for seeps with stygobionts.

Parameter	t	d.f.	Mean Difference	Corrected Sig. (2-tailed)	Uncorrected Sig. (2-tailed)
Temperature °C	0.028	69.8	-0.033	1.000	0.977
pH	1.599	58.7	0.415	0.922	0.115
Salinity (ppt)	0.214	72.1	-0.004	1.000	0.831
Conductivity (µS/cm)	0.229	68.4	7.573	1.000	0.819
Nitrate (ppm)	0.790	53.5	0.260	1.000	0.433
Nitrite (ppm)	0.249	59.6	-0.003	1.000	0.804
DO (mg/L)	0.584	75.8	0.373	1.000	0.561

Equation 1: Discriminant function for seeps with and without stygobionts incorporating six physicochemical variables using unstandardized coefficients.

$$2.776 - 0.093(Temp) - 0.429(pH) + 0.003(Cond) - 0.883(Nitrate) + 27.416(Nitrite) + 0.172(DO)$$

Equation 2: Discriminant function for seeps with and without stygobionts incorporating six physicochemical variables using standardized coefficients.

$$-0.409(Temp) - 0.452(pH) + 0.431(Cond) - 1.004(Nitrate) + 0.877(Nitrite) + 0.560(DO)$$

3.5 PHYSICOCHEMICAL CHARACTERISTICS OF SEEPS WITH AND WITHOUT *STYGOBROMUS*

Table 11 lists descriptives for the seven physicochemical parameters measured for seep sites in which *Stygobromus* species were and were not found. This data is visually represented by the boxplots in figures 36 through 42 which show mean values, quartiles, and outlying values for these seven parameters.

Table 11: Descriptive statistics for physicochemical parameters of seeps in which *Stygobromus* was and was not found.

Parameter	Stygobromus occurrence	N	Mean	Min	Max	Std. Dev.	Std. Error Mean	Coeff. Var.
Temp °C	Present	38	11.23	2.62	18.21	4.440	0.720	0.396
	Absent	52	11.52	0.05	22.50	5.860	0.810	0.509
pH	Present	37	6.042	4.3	8.11	1.058	0.174	0.175
	Absent	47	6.67	4.10	9.74	1.116	0.163	0.167
Salinity (ppt)	Present	33	0.10	0.02	0.34	0.090	0.020	0.975
	Absent	44	0.11	0.01	0.37	0.090	0.010	0.955
Cond (µS/cm)	Present	33	151.55	24.00	551.00	146.510	25.500	0.967
	Absent	45	158.48	9.16	673.00	141.810	21.140	0.895
Nitrate (ppm)	Present	28	1.11	0.00	5.00	1.530	0.290	1.371
	Absent	37	1.05	0.00	5.00	1.130	0.190	1.071
Nitrite (ppm)	Present	28	0.02	0.00	0.20	0.050	0.010	2.972
	Absent	37	0.01	0.00	0.15	0.030	0.000	4.330
DO (mg/L)	Present	38	6.31	1.99	14.06	2.530	0.410	0.401
	Absent	52	6.24	0.00	14.00	3.300	0.460	0.528

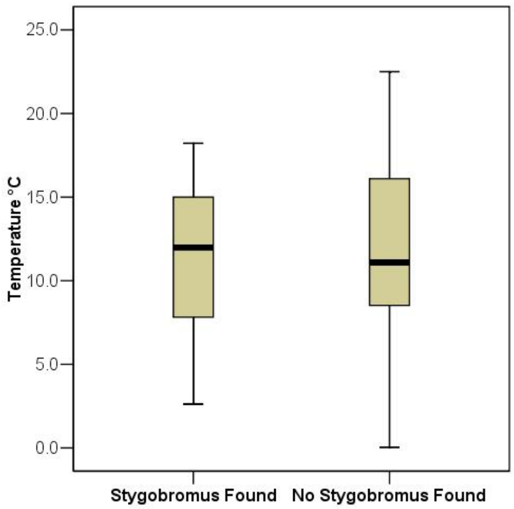

Figure 36: Boxplots depicting mean values, and quartiles for temperature of seeps where *Stygobromus* species were and were not found.

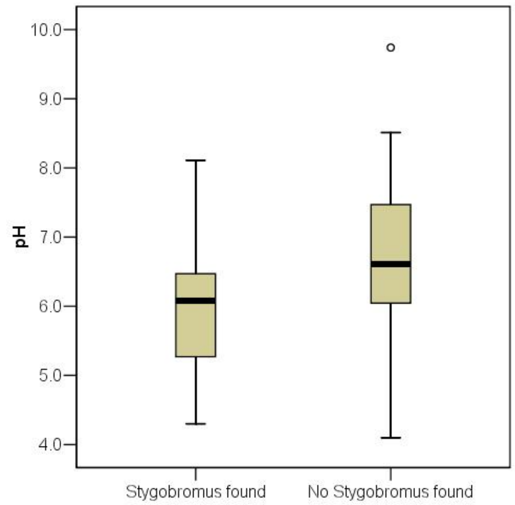

Figure 38: Boxplots depicting mean values, quartiles, and outlying values for pH of seeps where *Stygobromus* species were and were not found.

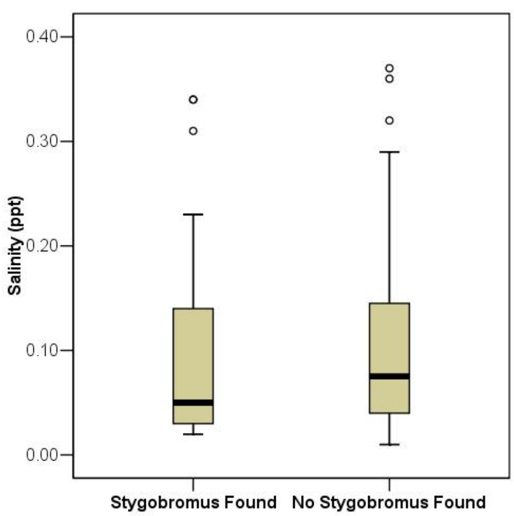

Figure 37: Boxplots depicting mean values, quartiles, and outlying values for salinity of seeps where *Stygobromus* species were and were not found.

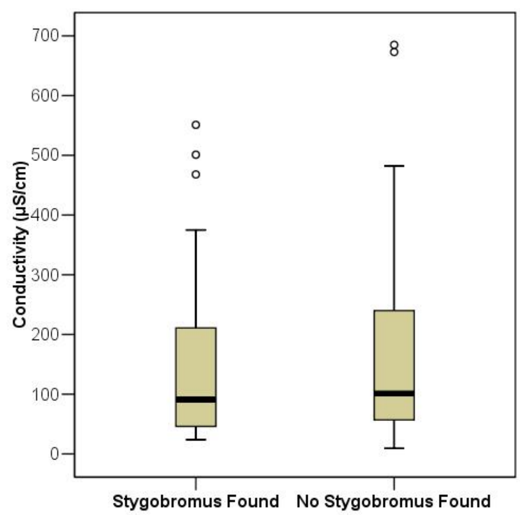

Figure 39: Boxplots depicting mean values, quartiles, and outlying values for conductivity of seeps where *Stygobromus* species were and were not found.

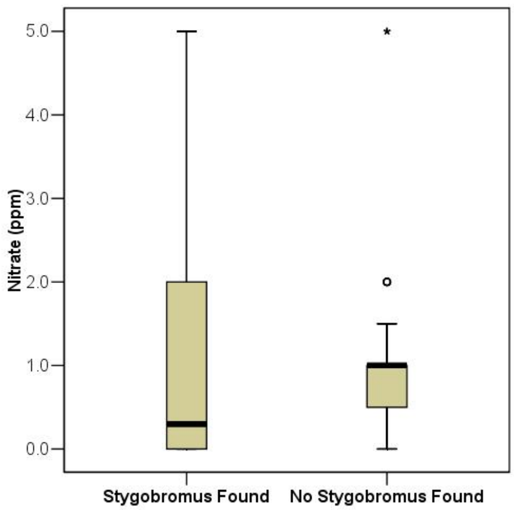

Figure 40: Boxplots depicting mean values, quartiles, outlying values, and extreme values for nitrate of seeps where Stygobromus species were and were not found.

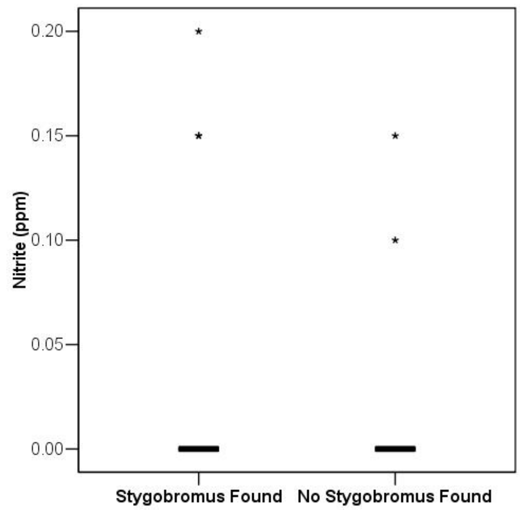

Figure 42: Boxplots depicting mean values, quartiles, and extreme values for nitrite of seeps where Stygobromus species were and were not found.

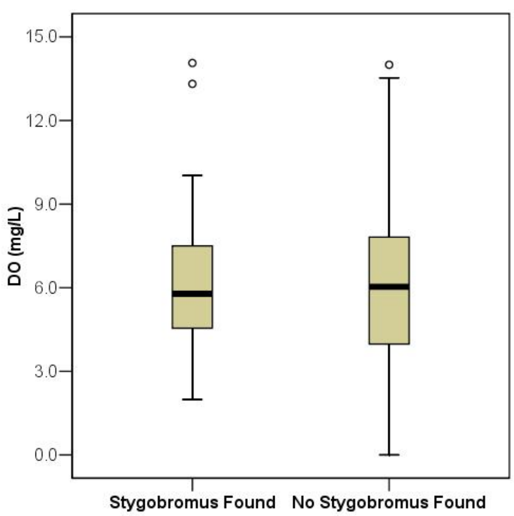

Figure 41: Boxplots depicting mean values, quartiles, and outlying values for dissolved oxygen in seeps where *Stygobromus* species were and were not found.

Table 12 depicts the results of seven Student's t-tests which tested for significant differences in the physicochemical parameters of seeps where *Stygobromus* species were and were not found. These tests revealed a significant difference between the mean pH of seeps with and without *Stygobromus*.

Discriminant analysis revealed that no variable or group of variables could be used to effectively discriminate between seeps with and without *Stygobromus* species. Using all six physicochemical variables, seeps with and without *Stygobromus* are different only at the $p = 0.87$ level (Wilks' lambda). Using six variables, the discriminant equation given in equations three and four successfully predict the absence of stygobionts 70% of the time and correctly predict the presence of stygobionts 55% of the time. Evaluated at the group means for each parameter, equations three and four equaled 0.348 for seeps without *Stygobromus* and -0.427 for seep with *Stygobromus*. This suggests that when evaluated for new seeps, these equations would yield negative values for seeps with *Stygobromus* and positive values for seeps without *Stygobromus*. Backwards stepwise exclusion resulted in the exclusion of all variables except pH (equation five). Using this equation, seeps with and without *Stygobromus* are different at the $p = 0.913$ level (Wilks' lambda). Equation five successfully predicts the absence of *Stygobromus* 52% of the time and correctly predicts the presence of *Stygobromus* 68% of the time. Evaluated at the group means for each parameter, equation five equals 0.274 for seeps without *Stygobromus* and -0.336 for seeps with *Stygobromus.* This suggests that when evaluated for new seeps, this equation would yield positive values for seeps without *Stygobromus* and negative values for seeps with *Stygobromus.*

Table 12: Results of Student's t-tests comparing mean values for seven physicochemical parameters between sites where *Stygobromus* species were and were not found. Mean difference = mean for seeps without *Stygobromus* – mean for seeps with *Stygobromus*.

Parameter	t	d.f.	Mean Difference	Corrected Sig. (2-tailed)	Uncorrected Sig. (2-tailed)
Temperature °C	0.271	87.9	0.295	1.000	0.787
pH	2.644	79.1	0.630	0.079	0.010
Salinity (ppt)	0.489	68.0	0.011	1.000	0.626
Conductivity (µS/cm)	0.209	67.8	6.938	1.000	0.835
Nitrate (ppm)	0.175	47.8	-0.060	1.000	0.861
Nitrite (ppm)	0.998	39.3	-0.011	1.000	0.324
DO (mg/L)	0.117	87.8	-0.072	1.000	0.907

Equation 3: Discriminate function for seeps with and without *Stygobromus* using unstandardized coefficients.

$$-6.052 + 0.084(Temp) + 0.838(pH) - 0.003(Cond) + 0.447(Nitrate) - 23.057(Nitrite) - 0.028(DO)$$

Equation 4: Discriminant function for seeps with and without *Stygobromus* using standardized coefficients.

$$0.374(Temp) + 0.854(pH) - 0.423(Cond) + 0.512(Nitrate) - 0.735(Nitrite) - 0.092(DO)$$

Equation 5: Discriminant function for seeps with and without Stygobromus using backwards stepwise removal of variables and unstandardized coefficients.

$$-6.494 + 0.982(pH)$$

3.6 DISCUSSION

Equations one through five, which were produced via discriminant analysis, can be used to predict whether stygobionts and *Stygobromus* species are present or absent in new seeps given chemical data. Specifically, when evaluated for new seeps, equations one and two yield positive values for seeps with stygobionts and negative values for seeps without stygobionts, equations three and four would yield negative values for seeps with *Stygobromus* and positive values for seeps without *Stygobromus*, and equation five yields positive values for seeps without *Stygobromus* and negative values for seeps with *Stygobromus.* However, these results should be interpreted with caution because of non-significant p values and high error rates for all five equations.

In general, t-tests revealed few significant differences between seeps and hyporheic sites, and seeps with and without stygobionts and *Stygobromus.* The major exception to this is pH which was found to be significantly higher in seeps without *Stygobromus* species. This result was also found by Culver and Chestnut (2003).

Of note, five seeps had detectable levels of nitrites. Three of these sites were in Difficult Run, George Washington Memorial Parkway. The other two sites were in Manassas National Battlefield Park and the southern portion of George Washington Parkway. Nitrite is a less stable, more toxic source of nitrogen formed from bacterial processing of ammonia which is ultimately converted to nitrate. Stygobionts, including *Stygobromus*, were found in three of these five sites suggesting that the presence of nitrite may not have a significantly adverse affect on the presence of stygobionts.

Discriminant analysis could not consistently discriminate between seeps with and without stygobionts and seeps with and without *Stygobromus*. This is in contrast with the findings of Culver and Chestnut (2006) who did similar analyses for 114 seeps and 94 hyporheic sites within the George Washington Memorial Parkway. This study found significant differences in several physicochemical parameters using the same analytical tools (Table 13). Furthermore, using discriminant analysis, Culver and Chestnut (2006) were able to correctly differentiate between seeps with and without stygobionts 90.9% of the time and seeps with and without *Stygobromus* 90.0% of the time. Variation in the physicochemical parameters of seeps due to regional differences may obscure differences between seeps with and without stygobionts and *Stygobromus.*

Consequently, differences between seeps with and without stygobionts or *Stygobromus* may have been obscured in our data which was collected over a much larger geographic area than that of Culver and Chestnut. Furthermore, the extent to which the physicochemical parameters of seeps vary seasonally is unknown. Collecting data during a short time span may be useful for better elucidating possible physicochemical parameters that explain the presence and absence of stygobionts and *Stygobromus* in seeps.

Table 13: Significantly different physicochemical parameters revealed for three comparisons from Culver and Chestnut (2006).

Seeps versus Hyporheic Sites	Seeps with and without Stygobionts	Seeps with and without *Stygobromus*
Temp °C Salinity (ppt) DO (mg/L) pH Nitrite (ppm)	Salinity (ppt) Nitrites (ppm)	Nitrites (ppm)

SIGNIFICANT AND IMPACTED SEEPS

Especially significant seeps were identified based on three criteria. These are seeps that should be considered priorities for management efforts. First, seeps which contain rare or endangered species are considered significant. Sites which contain multiple species of stygobionts, and especially those sites that contain multiple species of *Stygobromus* are considered significant. Finally, sites that contain large numbers of individuals of a single species of *Stygobromus* are considered unique. Seeps which show evidence of anthropogenic impact or could be at risk from potentially negative impacts are also discussed.

4.1 SIGNIFICANT SEEPS

4.1.1 George Washington Memorial Parkway

WR1, PIM2, GULF2 (Fig. 10, 11): These three sites each contained two species of *Stygobromus* in addition to *C. kenki*. The presence of three stygobionts and two species of *Stygobromus* is significantly diverse.

DIFR1, DIFR2 (Fig. 10, 11): These two sites contained *Stygobromus tenuis potomacus*, *Caecidotea kenki*, and *Fontigens bottimeri* making them diverse in terms of number of stygobionts.

TR2, PIM1 (Fig. 10, 11): These sites contained three species of *Stygobromus:* the highest number of stygobiontic amphipods known from a North American seep thus far. Furthermore, these sites also contained *C. kenki* making them especially significant.

Aside from these significant seeps, the Fort Hunt and Morningdale Lane area had a higher density of seeps containing *Stygobromus tenuis potomacus* than any other area identified in this study. This could indicate an extensive hypotelminorheic zone and a large population of *S. tenuis potomacus*. Furthermore, the potential for discovery of more seeps in this area is good. Of the seeps identified in GWMP, SG2 was the most unusual because of the volume of water it discharges and the manner in which water emerges like a small artesian spring (Fig. 10, 12, 43).

Figure 43: Significant seeps in southern George Washington Memorial Parkway. See Fig. 10 for reference map. Map by Tammy Stidham.

4.1.2 Manassas National Battlefield Park

SM1 (Fig. 14, 44): Despite being in an active agricultural field, large numbers of *Stygobromus tenuis potomacus* where consistently found except during the summer when the seep was dry. More *S. tenuis potomacus* were probably seen at this seep than any other sampled during this study.

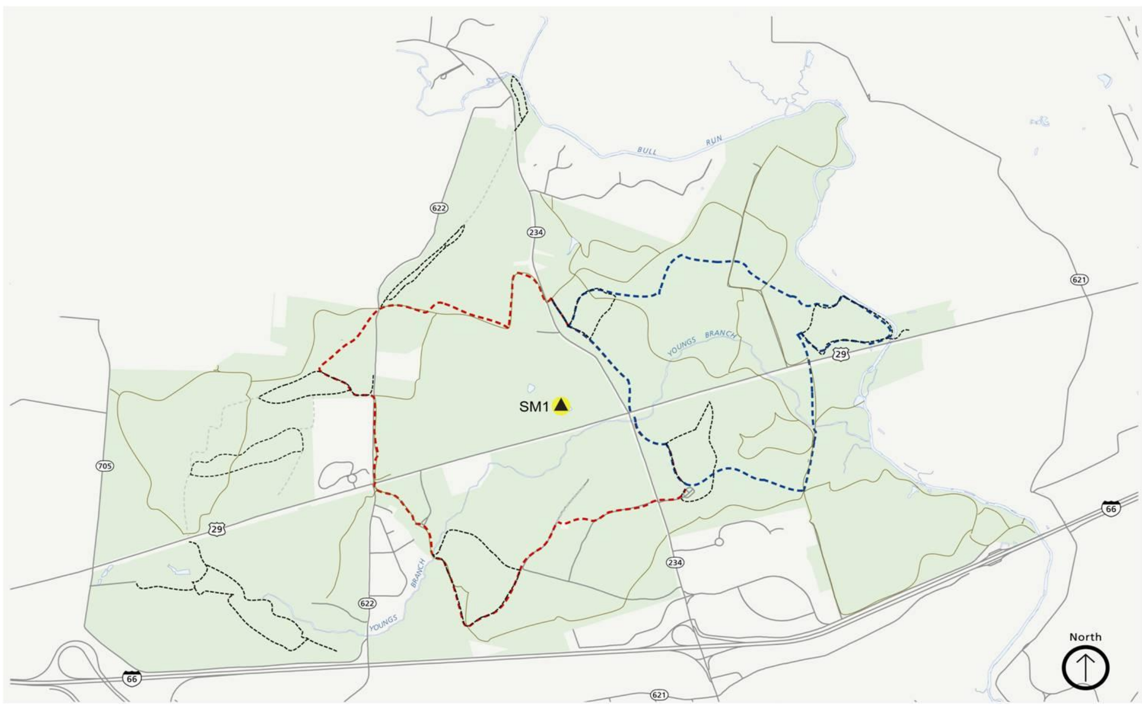

Figure 44: Significant seeps in Manassas National Battlefield Park. Map by Tammy Stidham.

4.1.3 National Capital Parks - East

Of all of the parks surveyed in this study, NACE probably has the best potential for discovery of additional seeps. Large parts of Oxon Run, Fort Chaplin Park, and some park lands between Fort Stanton Park and Dupont Park have not been searched. Some of these sites have unique habitats such as magnolia bogs, and seeps have been reported from some of these parks.

SE1 (Fig. 15, 45): This seep was probably the most significant seep in NACE because of its high density of *Stygobromus tenuis potomacus*.

Figure 45: Significant seeps in National Capital Parks - East. See Fig. 15 for reference map. Map by Tammy Stidham.

4.1.4 Prince William Forest Park

Like NACE, PRWI also has excellent potential for discovery of additional seeps. Because of its large area, several parks of PRWI were not thoroughly surveyed. All three sites where stygobionts were found in PRWI had very low densities of animals (no more than two *Stygobromus* where seen at any seep).

SP9 (Fig. 16, 46): This is the most significant site identified in this study because of the presence of an unidentified species of *Stygobromus*. Represented by a single individual, this species may be *Stygobromus sextarius* although more individuals are needed to make a positive identification.

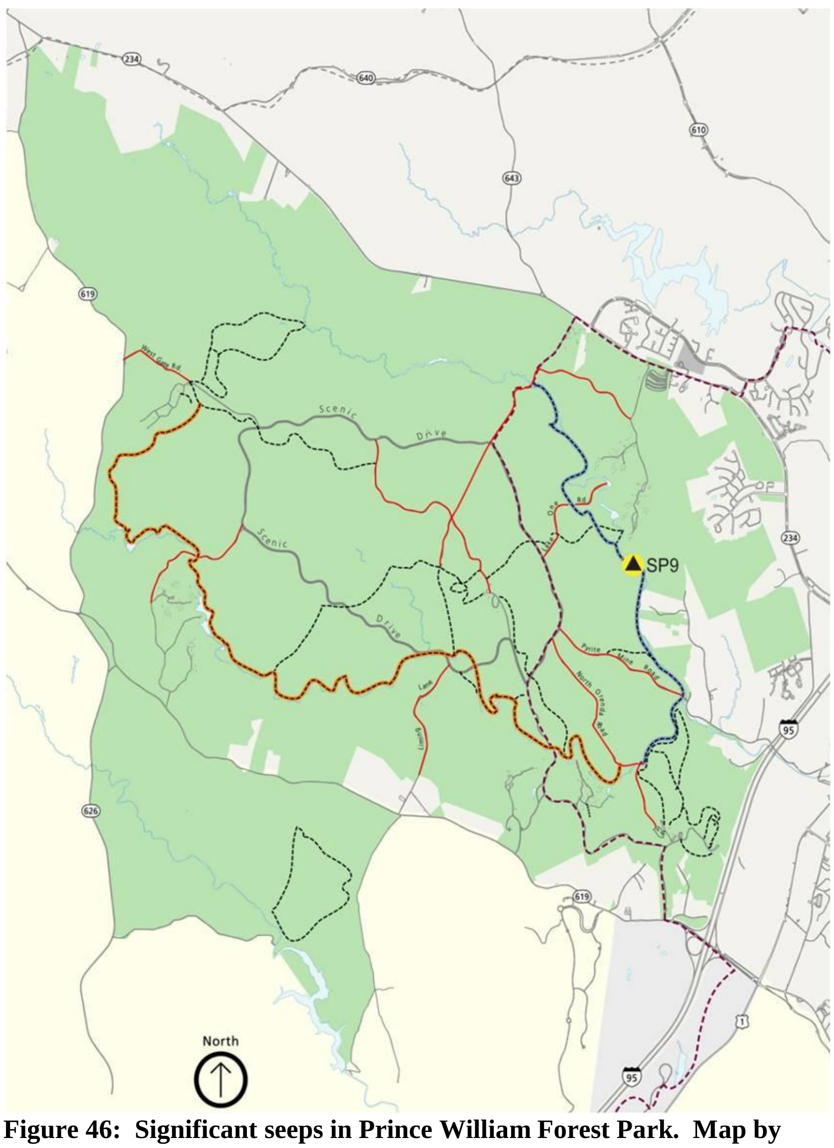

Figure 46: Significant seeps in Prince William Forest Park. Map by Tammy Stidham.

4.1.5 Wolf Trap Park for the Performing Arts

None of the Wolf Trap seeps had high densities or a high diversity of stygobionts. SW2 is probably the most significant seep because of its size (Fig. 17, 47).

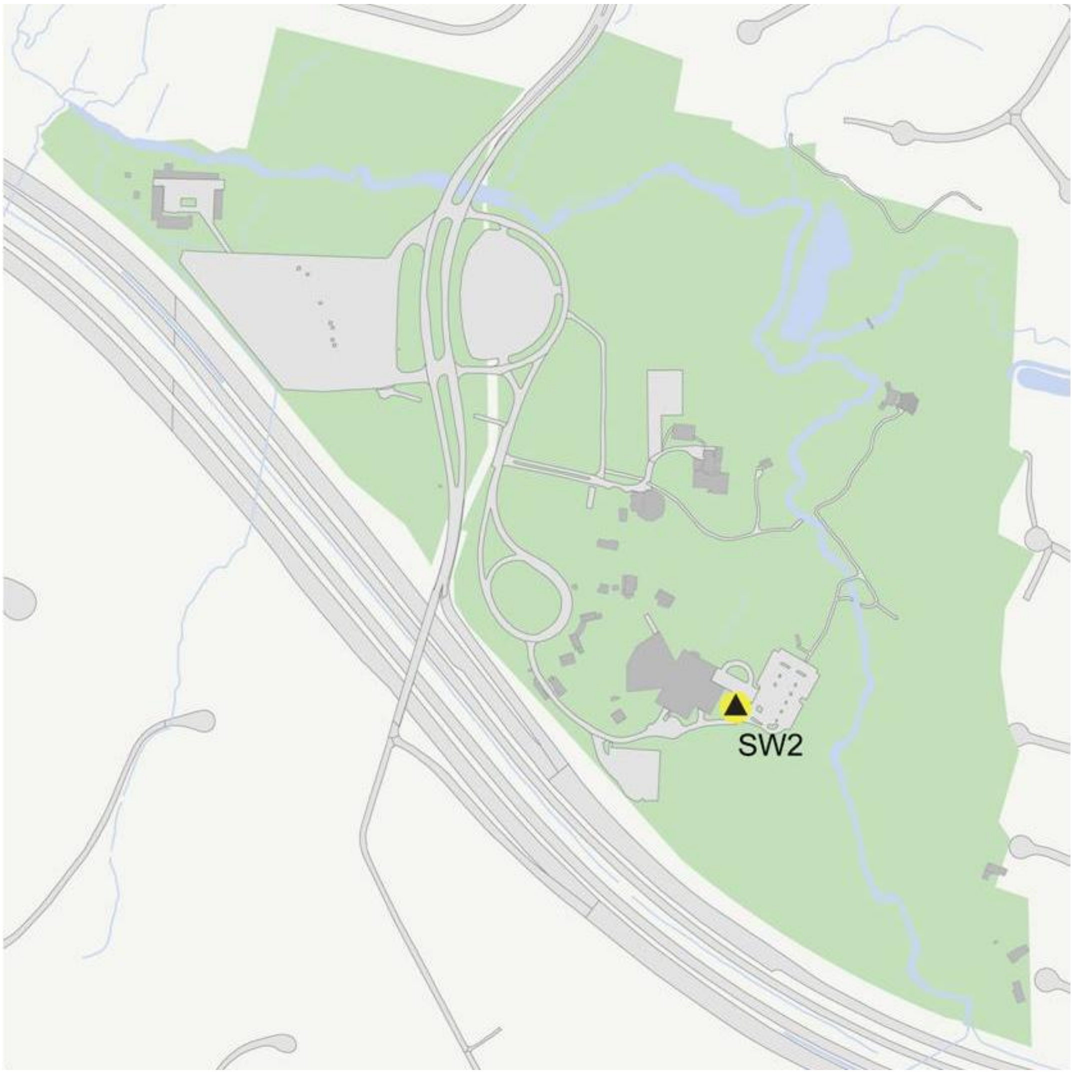

Figure 47: Significant seeps in Wolf Trap National Park for the Performing Arts. Map by Tammy Stidham.

4.1.6 Chesapeake and Ohio Canal National Historical Park

Although no single seep was especially diverse or contained a large density of stygobionts, the area around SC3, SC6 and SC7 was of particular significance because there was a high density of seeps (Fig. 18, 19, 48). Several additional, unsampled seeps in this vicinity were seen but not sampled and thus not included in this report. Importantly, *Stygobromus pizzinii* was found in most of these seeps. These seeps represent a previously undocumented population of this patchily distributed species.

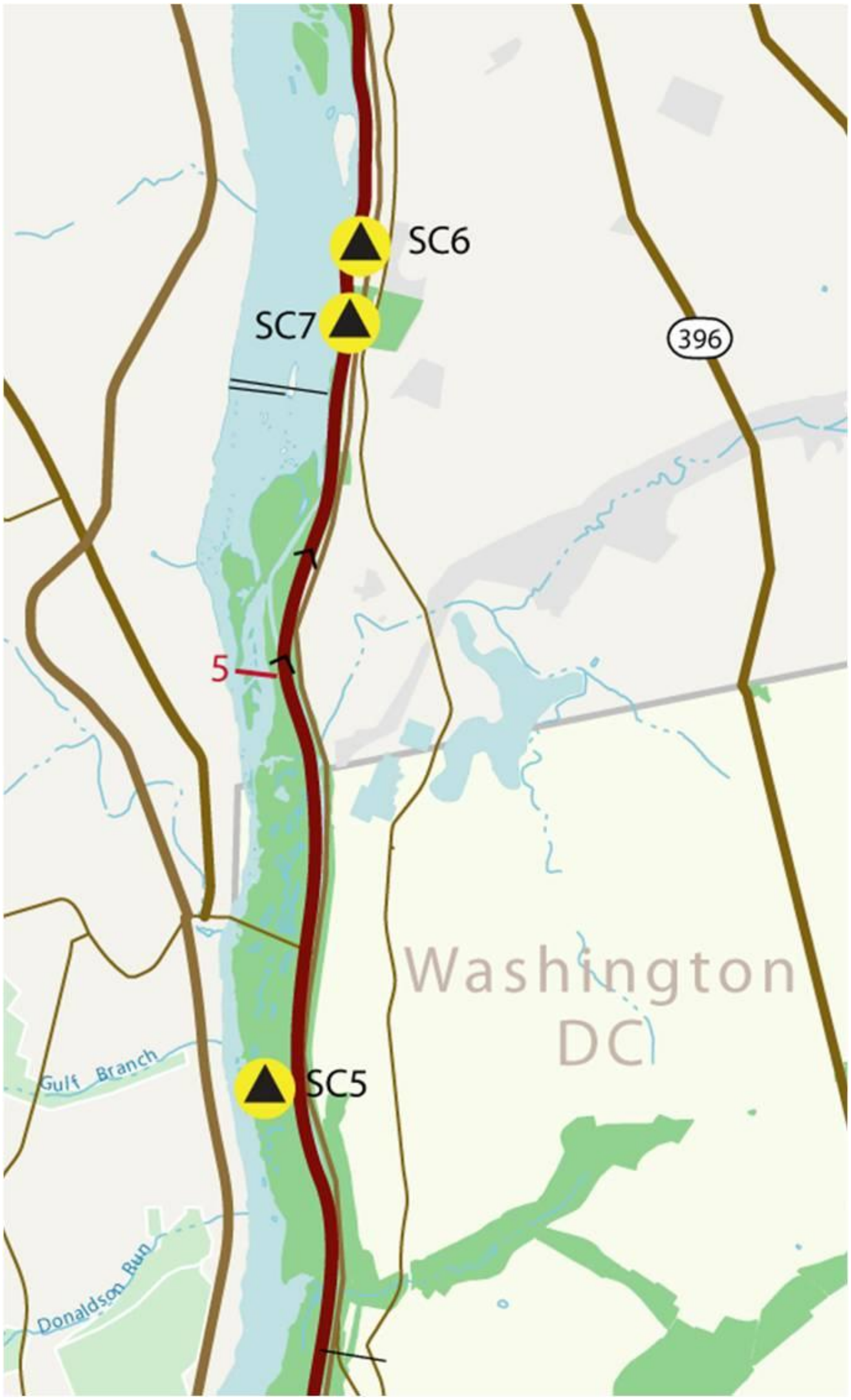

Figure 48: Significant seeps in Chesapeake and Ohio Canal National Historical Park. Seep SC3 not shown. See Fig. 18 for reference map. Map by Tammy Stidham.

4.1.7 Scott's Run Nature Preserve

SR1 and SR4 (Fig. 21, 49): These are especially significant seeps in Scott's Run because three species of stygobionts were found at these sites. Consequently, these are the most diverse sites, in terms of number of stygobionts, sampled during 2006 and 2007.

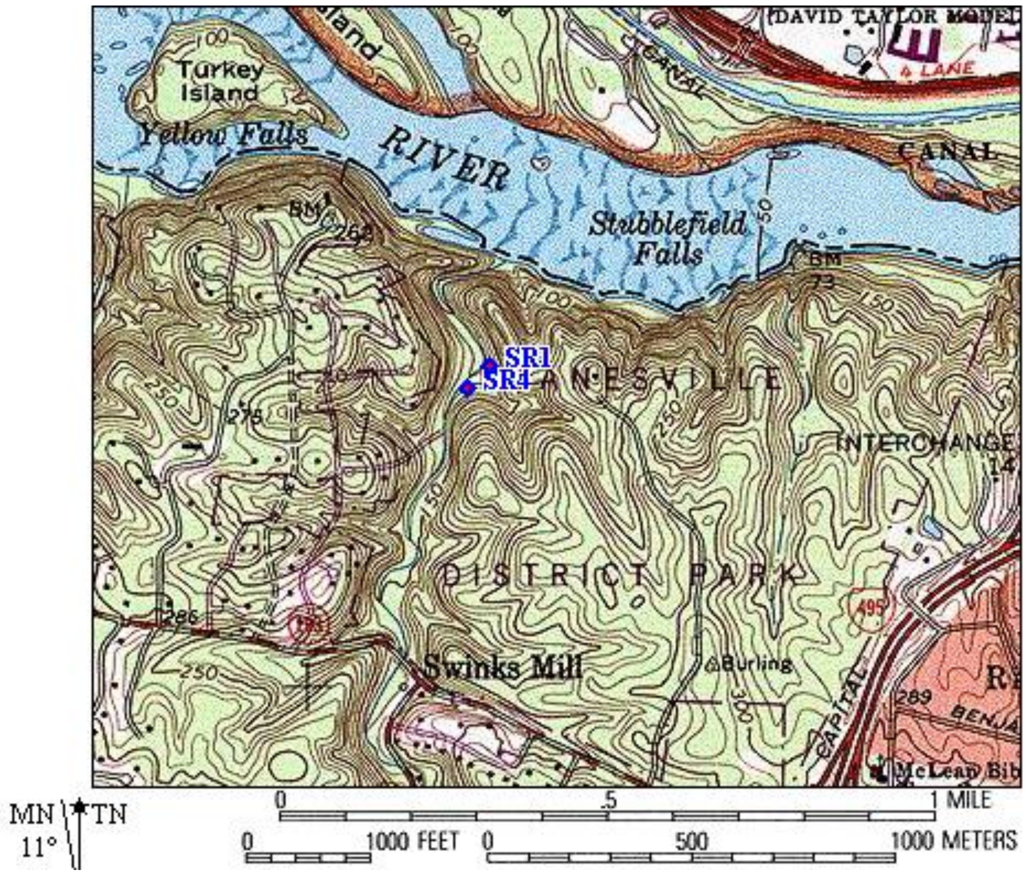

Figure 49: Significant seeps in Scott's Run Park. Map by Ben Hutchins.

4.2 IMPACTED OR POTENTIALLY IMPACTED SEEPS

4.2.1 George Washington Memorial Parkway (Fig. 1-13)

SG8: This seep contains *Stygobromus tenuis potomacus.* This seep is near the parkway in an area that is regularly mowed. Mowing equipment could compact the soil at this site. Furthermore, the site could be at risk of contamination from herbicide application. Leaving the area unmowed and avoiding the use of herbicide and pesticide would alleviate these risks.

SG11: This seep is down hill from the parkway and a paved trail. Runoff and salt as well as possible compaction from the trail may explain why no animals have been found at this promising seep. Salinity was found to be 0.37 ppt at this site. Although this is higher than most sites, *Stygobromus* has been found at sites with higher salinity.

SG12: This seep contains *Stygobromus tenuis potomacus.* This seep is near the parkway and could be contaminated by herbicide and pesticide application. Avoiding the use of herbicide and pesticide in this area would alleviate this risk.

SG13, SG14, and SG15: These seeps contain *Stygobromus tenuis potomacus.* These three seeps begin less than 2 meters from the parkway in a regularly mowed area. Mowing equipment and lack of vegetative cover put these seeps at risk from compaction and drying. Furthermore, herbicide or pesticide application and road salt could lead to contamination of these sites. Leaving this area unmowed would alleviate the risk of compaction and would allow for the growth of vegetation. Avoiding the use of herbicide or pesticide would alleviate the risk of contamination.

4.2.2 Manassas National Battlefield Park (Fig. 14)

SM1: This seep contains a large population of *Stygobromus tenuis potomacus.* The site is in a field and is at risk from compaction by farm equipment and contamination from fertilizer. Leaving this area unmowed and avoiding the use of fertilizer would alleviate this risk.

SM2: This seep contains *Stygobromus tenuis potomacus.* The site is partially wooded and partially in a field. Like SM1, it is at risk from compaction by farm equipment as well as water contamination from fertilizer.

SM5: This seep contains *Stygobromus tenuis potomacus*. This seep is in the riparian zone of a stream adjacent to a farmed field. The catchment for the seep appears to be primarily in the field, and contamination by fertilizer is possible. Avoiding the use of fertilizer would prevent contamination.

4.2.3 National Capital Parks - East (Fig. 15)

SE1: This seep contains *Stygobromus tenuis potomacus.* A sidewalk has been built over this seep several meters from its emergence point. Further down slope, the seep flows into an area that is regularly mowed and may be at risk of contamination from herbicide and pesticide application. A bridge over this seep and leaving the area unmowed would alleviate the risk of compaction. Avoiding the use of herbicides and pesticides would alleviate the risk of contamination.

SE4: This seep contains *Stygobromus tenuis potomacus.* This site is downhill from Oxon Hill Farm and could be at risk of contamination from agricultural runoff. Given the distance between the farm and the seep, this seems unlikely, but regular monitoring of water quality at this seep is advisable.

SE9 and 10: These sites are down slope from a large housing complex and parking lot. Runoff from the parking lot or groundwater contamination from the housing complex may explain why no animals have been found at these promising sites.

4.2.4 Prince William Forest Park (Fig. 16)

SP6: This seep contains an unidentified species of *Stygobromus*: potentially representing a significant range-extension for *Stygobromus sextarius* or a new species. The site is less than a meter down slope from a trail putting it at risk of soil compaction from foot traffic. Re-routing the trail or construction of a bridge over the seep would alleviate this risk.

4.2.5 Wolf Trap National Park for the Performing Arts (Fig. 17)

SW2: This seep contains *Stygobromus tenuis potomacus.* SW2 is a large seep a few meters from a parking lot, potentially putting it at risk of contamination from parking lot runoff. Insuring that runoff from the parking lot is not diverted into site SW2 would alleviate this risk

4.2.6 Chesapeake and Ohio Canal National Historical Park (Fig. 18-19)

SC6: This seep contains *Stygobromus pizzinii.* A culvert immediately uphill drains into this seep a few meters downstream from its' emergence. This culvert may put the seep at risk of contamination from road runoff including gasoline, salt, or heavy metals. Rerouting the culvert so that it does not drain into the seep would alleviate this risk.

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APPENDIX

Appendix 1: Coordinates (decimal degrees), elevation (ft.), and UTC date and time for seeps. Coordinates are in NAD83. Highlighted seeps were used in physicochemical analyses.

Site Name	Latitude	Longitude	Elevation	UTC-Date	UTC-Time
CIA1	38.95241	-77.14117	198	5/23/2007	13:48:27
DIFR1	38.97703	-77.24896	297	5/23/2007	13:48:50
DIFR2	38.97653	-77.24889	298	5/23/2007	13:49:41
DIFR3	38.97783	-77.24223	302	5/23/2007	13:50:08
DR1	38.96638	-77.17345	295	5/23/2007	13:36:50
DR2	38.96555	-77.17405	296	5/23/2007	13:37:18
DR3	38.96536	-77.1741	296	5/23/2007	13:37:42
FDR1	38.96518	-77.16414	273	5/23/2007	13:30:28
FDR2	38.96508	-77.16408	272	5/23/2007	13:31:15
GR1	38.99986	-77.25822	272	5/23/2007	13:56:52
GR10	38.98484	-77.25373	284	5/23/2007	14:00:33
GR11	38.98509	-77.25395	283	5/23/2007	14:00:59
GR2	38.99953	-77.25736	273	5/23/2007	13:57:17
GR3	38.99082	-77.25536	274	5/23/2007	13:57:42
GR4	38.98865	-77.25343	279	5/23/2007	13:58:03
GR5	38.98441	-77.25574	282	5/23/2007	14:01:23
GR6	38.98582	-77.2538	282	5/23/2007	13:59:47
GR7A	38.9859	-77.25375	282	5/23/2007	13:59:21
GR7B	38.98621	-77.25255	283	5/23/2007	13:58:58
GR8	38.98617	-77.25264	283	5/23/2007	13:58:32
GR9	38.98483	-77.25368	284	5/23/2007	14:00:08
GULF1	38.92306	-77.11579	156	5/23/2007	13:47:35
GULF2	38.92163	-77.11694	154	5/23/2007	13:47:57
PHT1	38.96681	-77.16403	274	5/23/2007	13:31:45
PHT2	38.9674	-77.16574	278	5/23/2007	13:32:29
PHT3	38.96607	-77.16771	282	5/23/2007	13:33:07
PHT4	38.96601	-77.16779	282	5/23/2007	13:33:51
PHT5	38.96586	-77.16782	282	5/23/2007	13:34:16
PHT6	38.96562	-77.16828	283	5/23/2007	13:35:00
PHT7	38.96567	-77.16966	286	5/23/2007	13:35:26
PHT8	38.96627	-77.17125	290	5/23/2007	13:35:54
PHT9	38.96639	-77.17187	292	5/23/2007	13:36:29
PIM1	38.92928	-77.11858	146	5/23/2007	13:46:51
PIM2	38.93052	-77.12047	142	5/23/2007	13:47:12
RB1	39.0137	-77.25005	184	5/15/2007	15:10:39
RB10	39.01418	-77.24858	162	5/15/2007	15:11:22
RB2	39.0159	-77.25536	267	5/15/2007	15:10:07
RB3	39.01526	-77.25485	254	5/15/2007	15:10:18
RB4	39.01458	-77.2535	242	5/15/2007	15:09:43
RB5	39.02606	-77.25265	188	5/15/2007	15:13:12
RB6	39.02717	-77.251	151	5/15/2007	15:13:45
RB7	39.02734	-77.25191	154	5/15/2007	15:13:35
RB8	39.02081	-77.2462	175	5/15/2007	15:12:35
RB9	39.02188	-77.24639	164	5/15/2007	15:12:16
SC1	38.95755	-77.13172	117	8/24/2007	17:05:06
SC2	38.95515	-77.13045	89	8/24/2007	17:06:44
SC3	38.9549	-77.12993	102	8/24/2007	17:07:26
SC4	38.93	-77.11248	28	8/24/2007	17:11:06
SC5	38.92452	-77.10994	7	8/24/2007	17:11:50
SC6	38.95469	-77.12988	103	8/24/2007	17:09:20
SC7	38.95326	-77.12876	105	8/24/2007	17:08:09
SE1	38.8947	-76.94307	81	7/9/2007	14:59:24
SE10	38.85126	-77.00462	67	7/9/2007	15:06:11
SE2	38.88037	-76.9494	108	7/9/2007	15:00:13
SE3	38.80615	-77.00929	73	7/9/2007	15:11:36
SE4	38.80446	-77.00921	83	7/9/2007	15:11:04

Appendix 1 continued.

Site Name	Latitude	Longitude	Elevation	UTC-Date	UTC-Time
SE5	38.80813	-77.00719	84	7/9/2007	15:15:40
SE6	38.8084	-77.00607	96	7/9/2007	15:16:08
SE7	38.84174	-77.00663	110	7/9/2007	15:10:35
SE8	38.84154	-77.00688	114	7/9/2007	15:07:26
SE9	38.851	-77.00402	59	7/9/2007	15:06:55
SG1	38.71376	-77.05413	NA	6/18/2007	15:23:23
SG10	38.71092	-77.05467	NA	6/18/2007	15:19:29
SG11	38.71652	-77.08366	NA	6/18/2007	15:21:53
SG12	38.76166	-77.05033	44	6/25/2007	15:19:14
SG13	38.75788	-77.049	8	6/25/2007	15:24:58
SG14	38.75569	-77.04984	37	6/25/2007	15:21:00
SG15	38.75537	-77.04955	23	6/25/2007	15:21:46
SG16	38.71315	-77.05412	39	6/18/2007	16:32:52
SG17	38.71509	-77.05188	NA	6/18/2007	15:23:58
SG2	38.71154	-77.05451	30	6/18/2007	16:31:52
SG3	38.716	-77.0543	48	6/18/2007	16:27:57
SG4	38.71441	-77.07687	20	6/18/2007	16:26:52
SG5	38.71564	-77.07916	NA	6/18/2007	15:22:48
SG6	38.71615	-77.08199	42	6/18/2007	15:54:27
SG7	38.7135	-77.05811	58	6/18/2007	16:30:10
SG8	38.7106	-77.05652	NA	6/18/2007	15:18:09
SG9	38.7109	-77.05506	NA	6/18/2007	15:18:51
SM1	38.8187	-77.53263	228	5/9/2007	17:01:51
SM2	38.82725	-77.53236	256	5/9/2007	16:59:26
SM3	38.82485	-77.50394	167	5/9/2007	16:56:36
SM4	38.81999	-77.51307	166	5/9/2007	16:57:03
SM5	38.81429	-77.54417	213	5/9/2007	17:01:19
SM6	38.80434	-77.52712	244	5/9/2007	17:00:01
SP1	38.55845	-77.34976	NA	5/9/2007	16:23:27
SP2	38.55826	-77.35669	NA	5/9/2007	16:28:40
SP3	38.56302	-77.36177	NA	5/9/2007	16:29:13
SP4	38.56094	-77.34496	NA	5/9/2007	16:27:15
SP5	38.59742	-77.37853	NA	5/9/2007	16:31:53
SP6	38.58564	-77.35469	NA	5/9/2007	16:30:24
SP7	38.60215	-77.41095	NA	5/9/2007	16:33:18
SP8	38.60745	-77.41021	NA	5/9/2007	16:34:02
SP9	38.58595	-77.35522	NA	5/9/2007	16:31:06
SR1	38.96546	-77.20284	147	5/15/2007	15:00:36
SR10	38.96683	-77.19603	122	5/15/2007	15:04:00
SR2	38.96189	-77.20403	209	5/15/2007	14:58:31
SR3	38.96148	-77.20419	178	5/15/2007	14:57:45
SR4	38.96501	-77.20347	115	5/15/2007	15:00:27
SR5	38.96677	-77.20043	86	5/15/2007	15:04:45
SR6	38.96677	-77.19948	71	5/15/2007	15:04:33
SR7	38.96739	-77.20274	81	5/15/2007	15:01:55
SR8	38.95937	-77.20531	176	5/15/2007	14:55:58
SR9	38.95967	-77.20541	175	5/15/2007	14:56:34
SW1	38.93702	-77.26431	292	5/14/2007	12:58:05
SW2	38.93622	-77.26381	288	5/14/2007	12:57:12
SW3	38.93766	-77.26249	298	5/14/2007	12:58:47
SW4	38.94103	-77.2661	256	5/14/2007	13:05:55
TR1	38.96118	-77.14332	217	5/23/2007	13:41:46
TR10	38.95863	-77.15766	251	5/23/2007	13:46:27
TR2	38.96307	-77.14583	226	5/23/2007	13:42:18
TR3	38.96555	-77.15299	247	5/23/2007	13:42:44
TR4	38.96555	-77.15311	247	5/23/2007	13:43:05
TR5A	38.9657	-77.15558	253	5/23/2007	13:43:32
TR5B	38.96314	-77.15775	255	5/23/2007	13:43:55
TR6	38.96096	-77.15862	256	5/23/2007	13:44:35
TR7	38.96039	-77.15864	255	5/23/2007	13:45:01
TR8	38.96027	-77.15872	255	5/23/2007	13:45:31

Appendix 1 continued.

Site Name	Latitude	Longitude	Elevation	UTC-Date	UTC-Time
TR9	38.95992	-77.15814	253	5/23/2007	13:45:59
WR1	38.95992	-77.14364	216	5/23/2007	13:38:08
WR2	38.95936	-77.1439	216	5/23/2007	13:38:44
WR3	38.95921	-77.14412	216	5/23/2007	13:39:09
WR4	38.95948	-77.1445	218	5/23/2007	13:39:36
WR5	38.95858	-77.14356	214	5/23/2007	13:40:03
WR6	38.95921	-77.14389	216	5/23/2007	13:40:45
WR7	38.96003	-77.14323	215	5/23/2007	13:41:10
WR8	38.96001	-77.14325	215	5/23/2007	13:51:06

Appendix II: Coordinates (decimal degrees), elevation (feet), and UTC date and time for hyporheic sites sampled in this study. Coordinates are in NAD83.

Site Name	Latitude	Longitude	Elevation	UTC-Date	UTC-Time
HM1	38.82079	-77.5133	165	5/9/2007	16:57:29
HM2	38.82092	-77.5134	165	5/9/2007	16:58:00
HM3	38.81723	-77.5303	176	5/9/2007	17:00:35
HC1	38.92689	-77.1137	0	8/24/2007	17:12:28
HC2	38.92689	-77.1137	0	8/24/2007	17:12:28
HC3	38.92748	-77.1143	0	8/24/2007	17:16:09
HG1	38.92031	-77.107	180	6/18/2007	15:17:19
HG2	38.92031	-77.107	180	6/18/2007	15:17:19
HE1	38.8786	-76.9484	106	7/9/2007	15:02:13
HE2	38.87853	-76.9483	106	7/9/2007	15:05:44
HP1	38.56769	-77.365	NA	5/9/2007	16:29:51
HP2	38.56769	-77.365	NA	5/9/2007	16:29:51
HP3	38.57637	-77.3762	NA	5/9/2007	16:42:07
HP4	38.57579	-77.3755	NA	5/9/2007	16:34:37
HW1	38.93807	-77.2632	275	5/14/2007	12:59:01
HW2	38.93807	-77.2632	275	5/14/2007	12:59:01